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Oxidative Stability of Ground Chicken Patties Enhanced with Rosemary Diterpene Phenols Relative to Ascorbic Acid

B. M. Naveena[1]*, M. Muthukumar[1], Rituparna Banerjee[1], V. A. Shaju[2] and C. K. Ramesh[2]

[1]ICAR- National Research Centre on Meat, Chengicherla, Hyderabad -500092

[2]Kancor Ingredients Limited, Angamaly South, Ernakulam, Kerala 683573

ABSTRACT

Present study was conducted to optimize natural phenols mixture which can yield the antioxidative effect equivalent to 500 ppm ascorbic acid (AA) in raw and cooked ground chicken patties stored under refrigeration. Purified rosemary extract (Oxikan-R) with 2.8% carnosic acid (CA) and 0.55% carnosol has been incorporated into ground chicken patties at 0.05% (low-dose), 0.18% (high-dose) and compared with 0.05% AA and non-treated control. Oxikan-R and AA are effective ($p< 0.05$) in limiting the lipid oxidation in both raw and cooked chicken patties. Protective effects of antioxidants in stabilizing the meat color through reduction ($p< 0.05$) of % metmyoglobin, % denatured myoglobin and improved redness scores (a*-value) were observed during storage. Reduction in total plate counts of chicken patties treated with Oxikan-R compared to control suggested the antimicrobial activity of Oxikan-R. Present study demonstrates the efficacy of Oxikan-R as a natural alternative to ascorbic acid.

Keywords: *Rosemary, Carnosic acid, Carnosol, Chicken Patties, Lipid Oxidation*

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INTRODUCTION

Meat processing operations viz, mincing, addition of salts, cooking and other processing prior to refrigerated storage disrupt muscle cell membranes facilitating the interaction of unsaturated lipids with substances such as non heme iron, accelerating lipid oxidation leading to rapid quality deterioration and development of rancidity. Lipid and myoglobin oxidation are interrelated and oxidation of one will exacerbate the other. This is even true in chicken where oxidizing lipids enhanced the myoglobin oxidation resulting in deterioration of color (Naveena et al. 2010). Poultry meat was reported to develop rancidity and warmed-over flavour very easily as poultry fat is highly unsaturated and often has low content of tocopherols. Consequently the meat industry has continuously sought to develop new formulations designed to extend shelf-life and improve food safety.

To inhibit oxidative deterioration, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, and tert-butyl hydroquinone (TBHQ) have been widely used as food additives in many countries. Recent reports reveal that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Prior 2004). In the meat industry, ascorbic acid (AA) has been widely used for increasing the shelf-life of meat and meat products. However, depending on its concentration, AA either promoted or inhibited lipid oxidation in muscle foods (Djenaneet al. 2001). Hence, there is an urgency to find alternate natural antioxidants of plant origin to replace these synthetic antioxidants. The Rosmarinus officinalisis one of the most consumed spices and is well documented in literature for its functional properties. Previous reports indicated that rosemary extracts retard lipid oxidation and prolong the shelf life of meat products (Georgantelis et al. 2007; Hussain et al. 2017). In addition, rosemary extracts have also showed antimicrobial effect (Angioni et al. 2004). The antioxidant activity of rosemary extracts is related to the presence of phenolic diterpenes, such as carnosic acid (CA) and its derivatives, carnosol, rosmadial, rosmanol, rosmanol isomers, and methyl carnosate, and phenolic acids such as rosmarinic acid (Linares et al. 2012). Previous in-vitro investigations have shown that the antioxidant activity of rosemary extracts is primarily associated with the amount of carnosoic acid and subsequently, the total amount of phenolic diterpenes present (Thorsen et al. 2003). Most of the researchers have identified carnosol, carnosic acid and rosmarinic acid as major constituents that contribute to the antioxidant activity of rosemary. The relationship between the concentration of CA and carnosol components affects their antioxidant and antibacterial activities (Jordan et al. 2012) and the antibacterial activity was improved when carnosol was the major diterpene component. In our earlier study (Naveena et al. 2013) we have demonstrated that oil soluble carnosic acid in liquid form had significantly higher antioxidative effect in ground chicken patties relative to water soluble rosmarinic acid in powder form.

At concentrations necessary for natural preservatives from spices and other plant-based additives to possess effective bioactivity, odor and flavor of the food may be negatively impacted. Therefore, it is a challenge before the meat processors to optimize the concentration of natural antioxidants and minimum dosage at which better lipid and color stability can be obtained. Considering the requirements of meat industries, the present study was envisaged to evaluate the antioxidant, antimicrobial and color stabilizing effect of oil soluble, natural antioxidant (Oxikan-R) against the synthetic water soluble antioxidant (ascorbic acid) in raw and cooked ground chicken patties during storage under refrigeration.

MATERIALS AND METHODS

Materials: Purified rosemary extract, Oxikan-R (2.8% carnosic acid + 0.55% carnosol; oil soluble liquid), was sourced from KANCOR Ingredients Ltd., Ernakulam, Kerala, India. The

*Corresponding author Email address: Naveena.BM@icar.gov.in

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ascorbic acid (AA) was procured from Sigma-Aldrich Chemical Co., Germany. All other chemicals were of reagent grade or greater purity. Fresh boneless and skinless chicken thigh and breast meat was obtained from a poultry processing plant (Sneha Poultries, Hyderabad, India), and chilled overnight at 4 °C. The chilled meat was then coarsely ground first through a 13-mm plate followed by 8-mm plate in a meat grinder (SCHARFEN, Model X70, 58413 Witten, West Germany). Freshly ground meat was used for the experiment. Chicken samples were procured on separate occasions to carry out each of the three replications.

Preparation of Chicken Patties: Freshly ground chicken samples were subdivided into 4 lots and assigned to one of the following four treatments: Control (meat without any antioxidant); Oxikan-LD (Oxikan-R, low-dose; 0.05% equivalent to 15 ppm total phenols); Oxikan-HD (Oxikan-R, high-dose; 0.18% equivalent to 54 ppm total phenols); and AA (ascorbic acid, 0.05%). All the antioxidant treatments were on fat weight basis and final concentrations (for Oxikan-LD, 1.5 mg phenols/100 g fat whereas, for Oxikan-HD 5.4 mg phenols/100 g fat). The final fat percentage was adjusted to 5% w/w assuming the fat level of 5% in most of the commercially available low-fat ground chicken products. Oxikan-R was dispersed in crude groundnut oil (i.e. the oil without any antioxidants procured directly from oil refinery) before addition, whereas ascorbic acid (AA) was dissolved in distilled water before use. Equal quantity of oil and or/water was added to other samples. Salt (1% w/w) was added to all the samples during mixing.

After mixing, minced chicken (80 g portions) was molded manually using glass petri-plates smeared with oil in the form of burger patties with uniform and smooth surface. These samples were designated as raw patties which will be analyzed later along with cooked patties. The molded patties (15 x 90 mm) were cooked in convection type still air oven (baking oven) fitted with a fan for circulation of hot air. The oven temperature was fixed at 160 ˚C and the patties were cooked for 25 min or till the internal core temperature of the patties reaches 71 ˚C measured by a probe thermometer inserted into geometric center of the patty. Here the patties were cooked by a circulating hot air. After cooling to room temperature the patties were weighed and aerobically packaged in a low density polyethylene pouches and stored at 4 ˚C for 0, 3, 6 and 9 days for raw patties and 0, 7, 14, 21 and 28 days for cooked patties respectively and analyzed for pH, thiobarbituric acid reactive substances (TBARS), % metmyoglobin, percent denatured myoglobin, instrumental color analysis, microbial quality and sensory attributes.

Analysis of Samples

pH and Cooking Yield: The pH of homogenized raw and cooked meat suspensions were recorded using digital pH meter (Thermo Orion, Model 420A+, USA). The weights of samples were recorded before (raw weight) and after cooking and the percent cooking yield was calculated.

Thiobarbituric acid reactive substances (TBARS): The TBARS value of raw and cooked chicken patties was determined by using the extraction method (Witte et al. 1970) and the TBARS values were calculated using a TBA standard curve and expressed in mg malonaldehyde/kg.

Determination of metmyoglobin and denatured myoglobin: Myoglobin was extracted from raw and cooked patties using a modified procedure of Warris (1979) by blending with 0.04 M phosphate buffer followed by centrifugation. The absorbance of filtrate was measured at 525, 572, and 700 nm using a UV-VIS spectrophotometer. The % metmyoglobin (%Met Mb) and percent denatured myoglobin (PMD) was calculated according to Trout (1989).

Instrumental color: Instrumental color analysis of raw and cooked patties was performed using a Hunter lab Miniscan XE Plus colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) during storage with 25 mm aperture set for illumination D65, 10˚standard observer angle. CIE a* (redness) was measured on the outer and internal surface of cooked chicken patties from five randomly chosen spots (Hunter and Harold, 1987).

Microbiological evaluation: For determination of microbial counts, 10 g of meat sample was homogenized with 90 ml of, 0.1% sterile peptone water. Serial 10-fold dilutions were prepared by diluting 1 mL of homogenate in 9 mL of 0.1% peptone water. Appropriate serial dilutions were duplicate plated (Pour plate method) with plate count agar for total plate count (TPC) and plates were incubated at 37 0C for 48 h.

Sensory evaluation: At day 0, cooked patties were served to a sensory panel which consisted of 8-10 experienced panel members (Keokamnerd et al. 2008). Sensory attributes, including color, flavor and overall acceptability were determined using 8 point descriptive scale where 8 = very acceptable; 7 = acceptable; 6 = moderately acceptable; 5 = slightly acceptable; 4 = slightly unacceptable; 3 = moderately unacceptable; 2 = very unacceptable; and 1 = extremely unacceptable. The off-odor was measured using 8-point scale, where 8 = none; 7 = just detectable; 6 = very mild; 5 = mild; 4 = mild-disctinct; 3 = distinct; 2 = distinct-strong; and 1 = strong.

Statistical analysis: All data were analyzed using SPSS (SPSS version 13.0 for windows; SPSS, Chicago, IL, SA). Cooking yield, PMD and sensory attributes were analyzed using one-way ANOVA. For all the remaining parameters a 4 x 4 factorial design with three replicates was employed for raw meat storage data, whereas 4 x 5 factorial design with three replicates was employed for cooked meat storage data, with treatments and storage time as main effects using two-way ANOVA. Each experiment was replicated thrice (n = 3). The least significant difference (LSD) was calculated at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate composition, pH and cooking yield: Total phenolic content of Oxikan-R was found to be 30 mg/g. Oxikan-R was characterized using liquid chromatography-mass spectrometry and contained 2.8% carnosic acid (CA) and 0.55% carnosol. The

pH of fresh chicken varied from 6.00-6.10. Ground chicken had 76.38±0.07% moisture, 20.86±0.23% crude protein, 3.10±0.26% fat and 1.20±0.21% ash. Total of 4 samples were prepared and analysed at each storage interval under each replication as mentioned earlier: 1. Control, 2. Oxikan-LD, 3. Oxikan-HD, 4. Ascorbic acid (AA). The cooking yield (Table 1) of ground chicken patties varied from 81.38±2.94% to 83.83±1.32% between control and treatments and did not differ significantly (p> 0.05). The pH values varied between 5.97±0.08 to 6.28±0.26 and 6.17±0.09 to 6.37±0.03 for raw and cooked chicken patties respectively during storage. Cooking had increased the pH of chicken patties by 0.2 units. However, neither the antioxidant treatment nor storage had any effect on pH of chicken patties. Keokamnerd et al. (2008) have reported an increase in pH of ground chicken thigh meat by 0.1 pH unit during 12 days of storage in samples treated with commercial rosemary preparations.

TABLE 1: Cooking yield and percentage denatured myoglobin (PMD) in cooked chicken patties treated with OXIKAN-R and Ascorbic acid

Treatments	Cooking yield (%)	PMD
Control	82.96 ± 2.01	73.30 ± 3.11c
Oxikan-LD	81.43 ± 3.88	39.86 ± 2.85a
Oxikan-HD	81.38 ± 2.94	52.90 ± 5.72b
	83.83 ± 1.32	49.03 ± 2.80b

Values are Means ± SD of 3 replications (n=3). Oxikan-LD, Oxikan-R low-dose; Oxikan-HD, Oxikan-R high-dose; AA, ascorbic acid

Effect on lipid oxidation: Significant (p< 0.05) increase in lipid oxidation as measured by thiobarbituric acid reactive substances (TBARS) from 0.241 to 0.578 mg malonaldehyde/kg raw and ground chicken patties was observed for control during storage (Fig. 1a). Antioxidant treated patties exhibited increase (p> 0.05) of TBARS values from 0.196 to 0.229 mg malonaldehyde/kg during storage. No difference (p> 0.05) was observed between Oxikan-LD, Oxikan-HD and AA treated patties; however, all the treated patties had lower (p< 0.05) TBARS values compared to control. Oxikan-LD, Oxikan-HD and AA treatment resulted in 63.6%, 67.6% and 49.85% reduction in TBARS values compared to control samples on 9th day of storage in raw patties.

For cooked patties, TBARS values increased (p< 0.05) in control from 0.543 on day'0 to 1.365 mg malonaldehyde/kg sample on day' 28 (Fig. 1b). In treated patties, TBARS values have increased during storage (0 to 28 days) from 0.286 to 0.881, 0.134 to 0.419 and 0.301 to 0.335 mg malonaldehyde/kg for Oxikan-LD, Oxikan-HD and AA samples, respectively. Antioxidant treatment is effective in inhibiting the lipid oxidation and controlling the maximum TBARS value below 1.0 throughout the storage period compared to control wherein the TBARS value exceeded 1.0 mg malonaldehyde/kg on 14th day of storage under refrigeration. Antioxidant treatments have resulted in 35.4%, 69.3% and 75.4% reduction in TBARS values in Oxikan-LD, Oxikan-HD and AA treated patties respectively during refrigerated storage.

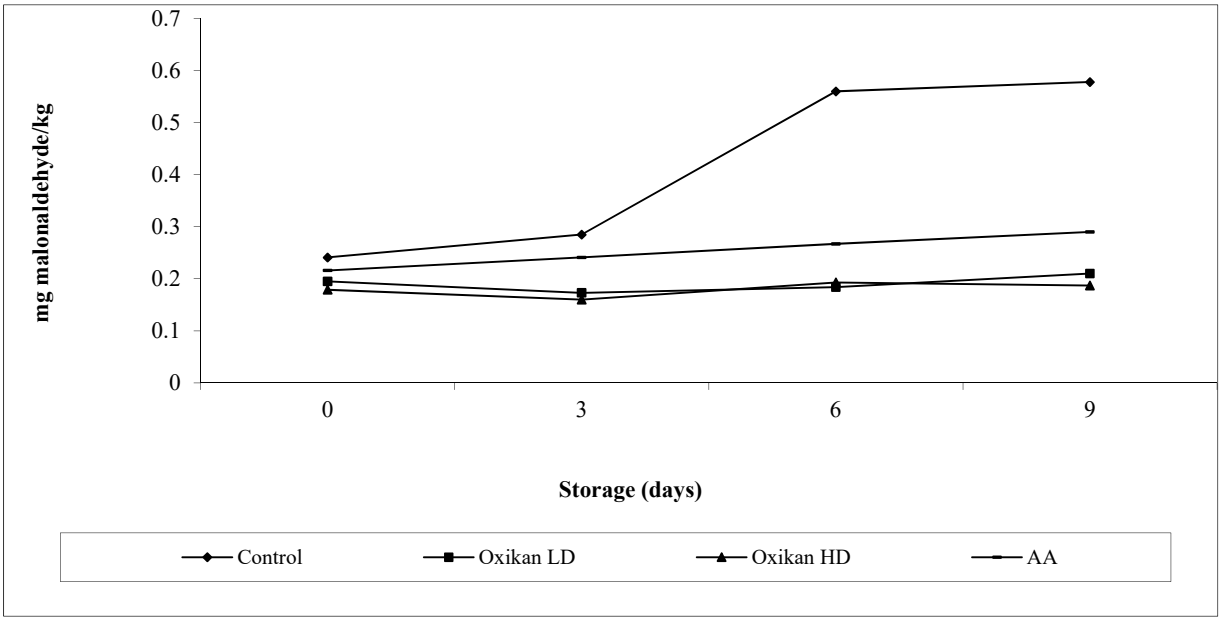

Fig. 1a: Effect of different levels of OXIKAN-R (Low-Dose, LD @ 0.05%; High-Dose, HD @ 0.18%) and Ascorbic Acid (AA, 0.05%) on lipid oxidation of raw ground chicken parries

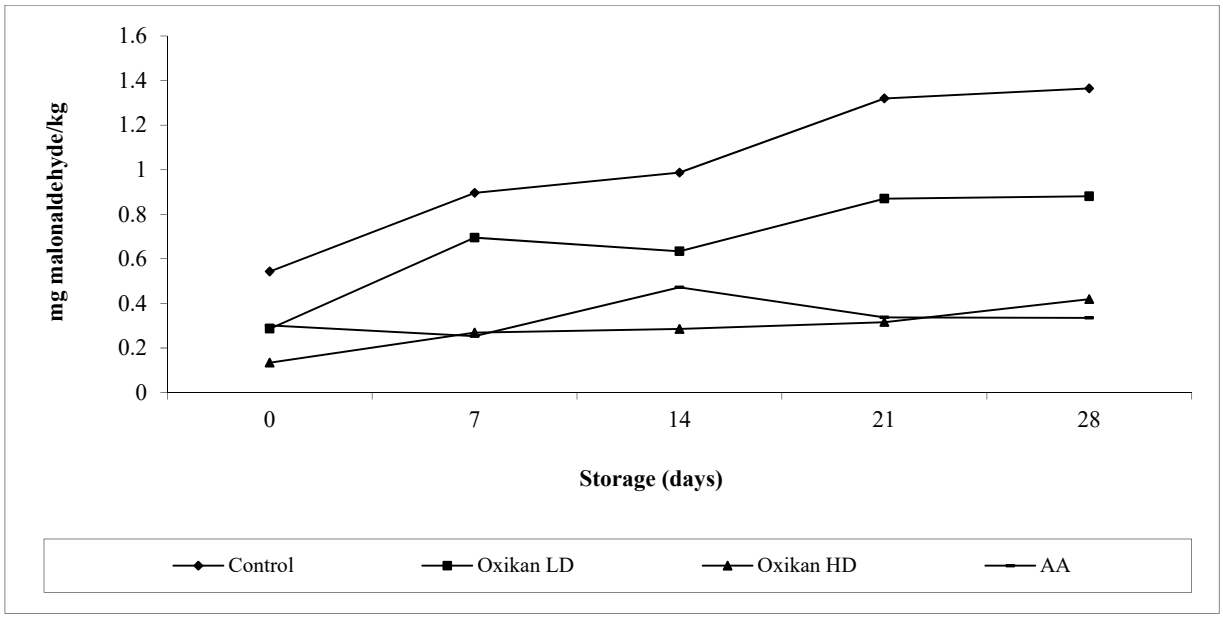

Fig. 1b: Effect of different levels of OXIKAN-R (Low-Dose, LD @ 0.05%; High-Dose, HD @ 0.18%) and Ascorbic Acid (AA, 0.05%) on lipid oxidation of cooked ground chicken patties

The strong antioxidant activity of phenolic diterpenes is due tothe action of hydrogen donator system which interferes with the free radical propagation process (Naveena et al.2011). The structure is important in providing hydrogen to donate which circumvents oxidation without creating another reactive free radical species. Moreover, several plant phenolic compounds also act as metal chelators and singlet O2 quenchers (Kasthuri et al. 2017). Ascorbic acid was also effective in inhibiting lipid oxidation compared to control throughout the storage. The antioxidant effect of AA was higher in cooked patties compared to raw patties wherein Oxikan-R had stronger effect. Addition of AA has been reported to significantly reduce the lipid oxidation in microwave cooked ground chicken patties during refrigerated storage (Naveena et al. 2007). In contrast to our findings addition of 500 ppm ascorbic acid in ground chicken patties was reported to be totally ineffective in inhibiting lipid oxidation and exhibited a prooxidant effect (Djenane et al.2001). Several authors have concluded that, when AA was used alone its antioxidant effect was limited, however when it is used in combination with other antioxidants it exerts a synergistic effect. But in the present study, Oxikan-LD, Oxikan-HD and AA are all effective in controlling lipid oxidation during storage of both raw and cooked ground chicken patties.

Effect on meat color: Addition of antioxidants significantly reduced ($p < 0.05$) the % metmyoglobin formation (Fig. 2) in raw ground chicken patties during storage compared to control.

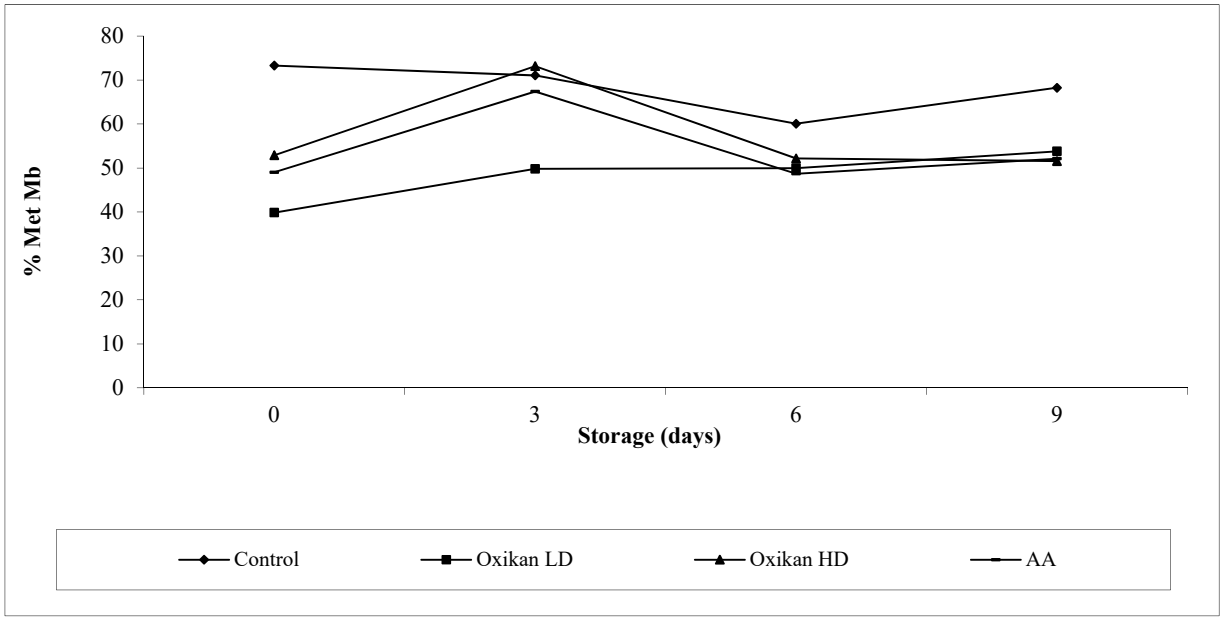

Fig. 2: Effect of different levels of OXIKAN-R (Low-Dose, LD @ 0.05%; High-Dose, HD @ 0.18%) and Ascorbic Acid (AA, 0.05%) on % Metmyoglobin content in raw ground chicken parries

These results correlate with higher ($p< 0.05$) Hunterlab a* (redness) (Fig. 3) values in all antioxidant treated raw patties especially on 6th and 9th day of storage compared to control samples. For raw patties, maximum increase in redness scores was observed in the order: Oxikan-HD>AA>Oxikan-LD>Control. Similar results were reported earlier where fresh chicken sausages incorporated with rosemary extract showed significant increase in redness scores during refrigerated storage (Liu et al. 2009). In cooked patties, higher ($p> 0.05$) surface redness scores (Fig. 4) were observed in Oxikan-HD and AA treated samples compared to control and Oxikan-LD sample. Significant reduction ($p< 0.05$) in %denatured myoglobin (PMD) was observed in all antioxidant treated patties relative to control. Oxikan-LD patties had lowest ($p< 0.05$) PMD values compared to Oxikan-HD and AA patties (Table 1).

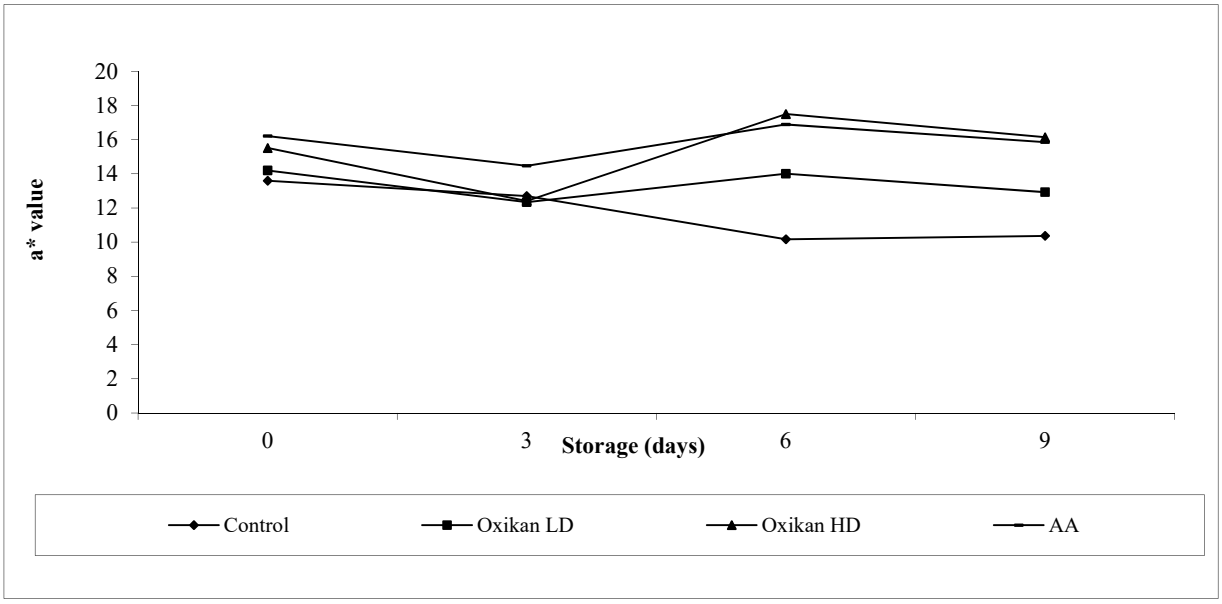

Fig. 3: Effect of different levels of OXIKAN-R (Low-Dose, LD @ 0.05%; High-Dose, HD @ 0.18%) and Ascorbic Acid (AA, 0.05%) on surface redness scores (a*value) of raw ground chicken patties

The state of the myoglobin in the interior of chicken patties at the time of cooking had major effect on development of cooked meat color. If patties contained predominantly MetMb a brown, morewell-done appearance would develop at the end point temperature (Hunt et al. 1999). These authors have further concluded that, MetMb and OxyMb are more thermos-labile, whereas DeoxyMb was always more heat stable. Our findings with ground chicken patties indicate that, control samples with higher MetMb had very high PMD, whereas, in antioxidant treated patties we expect that higher OxyMb or DeoxyMb would have resulted in lesser PMD.

Effect on Microbial Quality: Microbial counts of cooked samples (Fig. 4) as indicated by total plate counts (TPC) remained lower

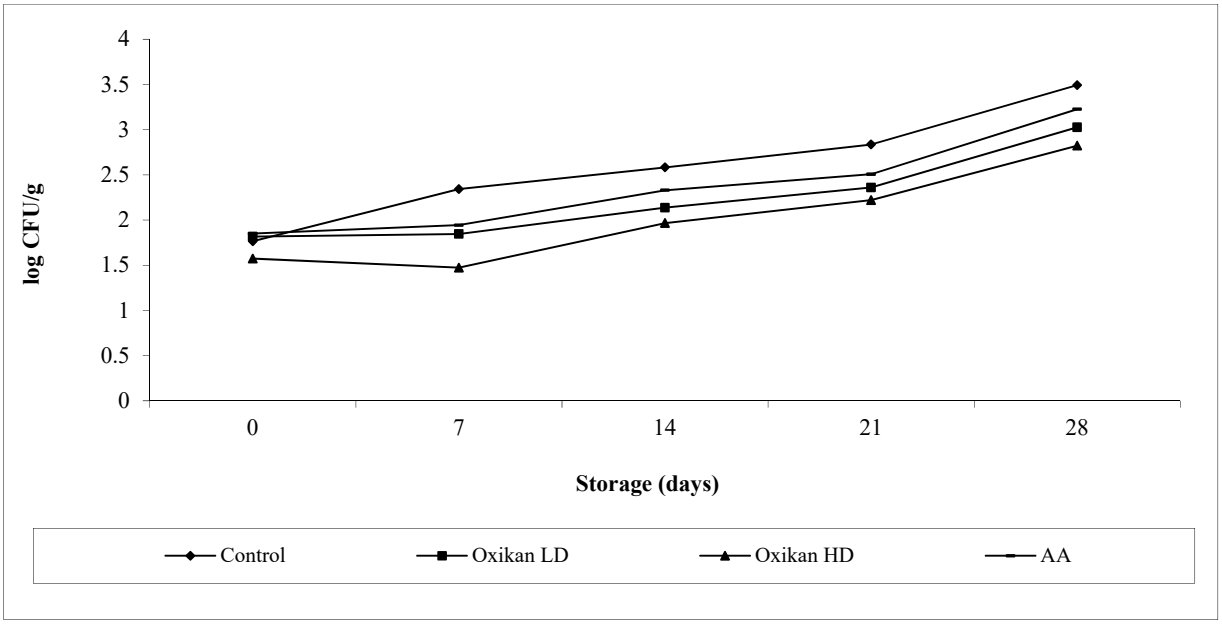

Fig. 4: Effect of different levels of OXIKAN-R (Low-Dose, LD @ 0.05%; High-Dose, HD @ 0.18%) and Ascorbic Acid (AA, 0.05%) on Total Plate Counts of cooked chicken patties

(p< 0.05) in Oxikan-HD samples compared to control and AA treated samples. The TPC increased (p< 0.05) from 1.766 cfu/g on day'0 to 3.493 cfu/g on day'28 for control and 1.850 cfu/g on day'0 to 3.226 cfu/g on day'28 for AA treated samples. For Oxikan-HD treated patties, the TPC increased (P> 0.05) from 1.573 cfu/g on day'0 to 2.823 cfu/g on day'28. Our results indicated antimicrobial activity of rosemary extract (Oxikan-R) in cooked chicken patties under refrigerated storage condition. Significant reduction in total plate counts of fresh chicken sausages incorporated with rosemary extract has been reported by Liu et al. (2009). Antimicrobial activity of non-polar components such as phenolic diterpenes against Gram-positive bacteria was also reported by (Fernandez Lopez et al. 2005).

Effect on Sensory Quality: Treatment with antioxidants increased (p> 0.05) the color, flavor and overall acceptability scores compared to control. No off-odor was detected in any of the antioxidant treated samples throughout the storage except a slight spice or herb like aroma in Oxikan-HD samples. However, warmed-over flavor was very much evident in control patties during storage (data not presented).

CONCLUSIONS

This study showed that, purified rosemary extract (Oxikan-R) with 2.8% carnosic acid and 0.55% carnosol was effective in inhibiting lipid oxidation in raw and cooked ground chicken patties and its antioxidant efficacy was equivalent to 500 ppm ascorbic acid. Oxikan-R was also efficient in protecting meat color and exhibited antimicrobial activity during storage. Use of Oxikan-R has no adverse effect on any of the sensory attributes of chicken patties. Therefore, it is concluded that Oxikan-R at 0.05% can be a natural alternative to 0.05% AA in ground chicken patties.

COMPETING INTERESTS: The authors have no known competing interests either financial or personal between themselves and others that might bias the work.

ETHICS STATEMENT: Not applicable

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