Effect of aerobic and modified atmosphere packaging on quality characteristics of chicken leg meat at refrigerated storage

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ABSTRACT The demand for chicken meat is continuously increasing in the consumer market. Increasing the shelf-life of chicken meat with modern packaging technology in the supply chain is necessary. Hence research was undertaken to study the effect of aerobic packaging (**AP**) and modified atmosphere packaging (**MAP**) on the quality and shelf-life of chicken meat. The chicken leg meat (**CLM**) was stored under refrigerated storage (4 ± 1°C) in aerobic and modified atmosphere packaging (MAP20 = 20%O_2 + 30%CO_2 + 50%N_2, MAP10 = 10%O_2 + 40%CO_2 + 50%N_2, MAP0 = 0%O_2 + 20%CO_2 + 80%N_2) conditions and evaluated for quality attributes. The results have indicated that MAP of chicken leg meat significantly increased the headspace carbon dioxide, Warner-Bratzler shear force value, standard plate count, color, and odor but decreased the TBARS value, headspace oxygen, and nitrogen when compared with AP. The pH, myoglobin forms, meat pigment, heme iron, CIELAB color space (L*, a*, b*), yeast and mold count, appearance, and sliminess were not affected significantly by AP and MAP. It is concluded that under refrigerated storage conditions, MAP extends the shelf-life of chicken leg meat up to 15 d compared to only 6 d for aerobic packaging.

Key words: chicken leg meat, aerobic packaging, modified atmosphere packaging, shelf-life, quality

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INTRODUCTION

Chicken meat is one of the most desirable meats due to its low price and good nutritive value because of the presence of high-quality protein, low amount of fat, high amount of unsaturated fatty acids, and relatively less saturated fatty acids. One method of controlling food quality and safety is the application of new packaging systems, which include active packaging. Modified atmosphere packaging is generally employed in the food industry to preserve the quality and prolong the shelf-life of meat and meat products. Mc Millin et al. (2008) stated that modified atmosphere packaging is the replacement and/or removal of the atmospheric gases surrounding the food product before sealing the package with vapor-barrier packaging materials.

The key principle of MAP is the exclusion of oxygen (which limits the shelf-life of meat by causing lipid oxidation and/or by increasing the growth of spoilage microorganisms) by using a barrier film or by altering the gaseous environment surrounding the meat. The use of any preservation method intended to improve the shelf-life of foods has to consider the dynamics of the total system. In the case of MAP-meat, the chief concerns as a result of dynamic changes are enzymatic ageing, microbial deterioration, oxidative rancidity, and differences in the oxidative forms of the myoglobin pigment (Narasimha Rao and Sachindra, 2002). Three gases are mainly used in MAP namely carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2). Other gases used in traces are carbon monoxide, sulfur dioxide, and nitrous oxide. Oxygen preserves the bright red color of meat but causes oxidative rancidity, growth of aerobic spoilage organisms, and premature browning during cooking. Carbon dioxide has an antimicrobial effect but it causes pack collapse and a minor decrease in pH. The efficiency of the MAP in improving the shelf-life of meat relies on the antibacterial property of carbon dioxide existing inside the package (Karabagias et al., 2011). Nitrogen is used as filler gas as well as to prevent pack collapse caused by carbon dioxide. Nitrogen has no antimicrobial properties and does not affect the meat color. Carbon monoxide (**CO**) has been very effective in maintaining the red color in fresh meat due to the formation of carboxymyoglobin. CO does not affect bacterial

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growth. However, due to its toxicity, it has not been approved by the regulatory agencies, except in Norway (Narasimha Rao and Sachindra, 2002).

The Indian poultry industry is valued at 18.5 billion USD. Poultry meat production constitutes 50% of India's total meat production. The annual production of chicken meat in India is 4.06 million tonnes with an annual growth rate of 8% (DAHD, 2020). The population of India is around 1.30 billion growing at 1.04%. In 2020, the annual per capita consumption of chicken meat was 3.5 kg against the Indian Council of Medical Research (**ICMR**) recommendation of 10.5 kg of chicken meat, a way well below the recommendation. About 1.74 million tonnes of poultry slaughter waste is produced annually (APEDA, 2020). The volume of poultry business in India needs scrupulous implementation of innovative technologies in every aspect of meat processing, packaging, and distribution. In developing countries like India, meat and meat products are prepared from a wide range of food animals including poultry. Hence, it is necessary to develop meat and product type-specific gaseous combinations for MAP. This would enhance the shelf-life, and maintain the nutritive value of meat and its products for an extended period compared to the conventional packaging. Besides, there is inadequate research on MAP in India, more specifically on the comparison of different gaseous compositions for chicken leg meat and the effect of aerobic packaging versus MAP on chicken meat quality. Therefore this research was undertaken to study the comparative effects of MAP and aerobic packaging on the quality attributes of chicken meat.

MATERIALS AND METHODS

Meat Sample

The chicken meat was collected from the local market of Hyderabad, India. The chickens were slaughtered as per the ethical guidelines outlined in IS 4674: 1975 for dressed chicken issued by the Bureau of Indian Standards. In each trial, 24 leg pieces (8 for AP and 16 for MAP) of chicken meat cut were utilized considering duplicate samples for each group for each storage day. In total 72 leg cuts of chicken meat were used in the entire experiment in three trials. Each leg cut was packaged separately in trays. So 72 package of chicken leg cut was utilized during the entire storage study. The chicken leg meat sample of approximately 200 g was weighed and placed in a clean tray (Tray-EVOH; Overwrap-PET/PP). The film characteristics of EVOH used for the tray were oxygen permeability of 0.5 $cm^3/m^2/24$ h, and water vapor permeability of 1,000 $g/m^2/24$ h. The characteristics of overwrap film consisting of PET/PP were oxygen permeability of 1,500 $cm^3/m^2/24$ h, and water vapor permeability of 15 $g/m^2/24$ h. The packaging trays were sterilized under the UV chamber for 30 min to avoid any cross-contamination. For modified atmosphere packaging, the gas mixture (O_2, CO_2, N_2) was blended in a Gas mixing machine (Elixir technologies, GAS MIXER - E2M316, Bangalore) attached to oxygen, carbon dioxide, and nitrogen cylinders. Then the trays were gas flushed and sealed in a Tray sealing machine (Elixir technologies, Tray sealer -ETS 300 GS, Bangalore). The gas concentrations used in modified atmosphere packaging were MAP-20 ($20\%O_2 + 3\ 0\%CO_2 + 50\%N_2$), MAP-10 ($10\%O_2 + 40\%CO_2 + 50\%N_2$), and MAP-0 ($0\%O_2 + 20\%CO_2 + 80\%N_2$). In aerobic packaging (**AP**), the trays were sealed using a tray sealing machine without flushing any gas. The packaged meat was then stored under refrigeration storage at 4 ± 1°C. The aerobically packaged meats were analyzed on 0, 3, 6, and 9 d of storage. Modified atmosphere packaged samples were studied at 0, 3, 6, 9, 12, 15, 18, and 21 d of storage.

Physico-chemical Parameters

Proximate Composition The moisture content was estimated by drying method in a hot air oven, protein using automatic digestion and distillation unit, and the fat was estimated by ether extraction following AOAC (1995).

pH The pH of the chicken meat sample was estimated using the portable handheld pH meter (Hannah Instruments, H198163, Romania). The probe is provided with a stainless steel conical blade and conical glass electrode, which was cleaned using the electrode cleaning solution. The pH meter was calibrated using 2 buffer solutions (pH = 4.0 and pH = 7.0).The probe was inserted at 5 different areas in the meat sample and the pH values of 5 readings were recorded.

Thiobarbituric Acid Reactive Substances (TBARS) The thiobarbituric acid reactive substances method was used to determine the lipid oxidation in chicken meat. Zhang et al. (2019) method was used with slight modification. About 2.5 g of chicken meat sample after trimming off the fat and connective tissue was taken. It was homogenized with 12.5 mL of distilled water in an Ultrasonic probe sonicator (PCI Analytics, PKS-750F, Mumbai, India) for 1 min. About 12.5 mL of 10% w/v Trichloroacetic acid (**TCA**) was added. The mixture was vortexed for 1 min and then filtered through filter paper grade No.1 (Hi-Media Laboratories Pvt Ltd., Mumbai**).** Four milliliter of the filtrate was collected in a test tube and 1 mL of 0.06 M Thiobarbituric acid (**TBA**) was added. Test tubes were incubated in the water bath at 80°C for 90 min. Using a UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan), the absorbance was recorded at 532 nm. Two milliliter of distilled water + 2mL of 10% TCA + 1 mL of 0.06 M TBA, was set as blank. Results were interpreted as TBARS in mg malondialdehyde (**MDA**)/ kg chicken meat.

Myoglobin Content To extract the myoglobin from the chicken meat sample Krzywicki (1982) and Shang et al. (2020) method was used. Five gram of chicken meat sample was weighed and then 50 mL of phosphate

buffer (40 mmol/L, pH 6.8, 4°C) was added to it. Using an Ultrasonic probe sonicator (PCI Analytics, PKS-750F, Mumbai, India) the samples were homogenized and kept in an ice bath for 60 min. Then the samples were centrifuged in a refrigerated centrifuge machine (4° C) for 30 min at 5,000 rpm. The supernatant was filtered through filter paper Grade no: 1 (Hi-Media Laboratories Pvt Ltd., Mumbai**).** Krzywicki's equations were modified using wavelength maxima at 503 nm, 557 nm, and 582 nm for metmyoglobin (**MMb**), deoxymyoglobin (**DMb**), and oxymyoglobin (**OMb**) respectively. Absorbance was recorded using a UV-VIS spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan), at 525 nm, 503 nm, 557 nm, and 582 nm. The proportions of the three forms of myoglobin were calculated as follows:

$$\mathrm{DMb\%} = -0.543R_1 + 1.594R_2 + 0.552R_3 - 1.329$$

$$\mathrm{OMb\%} = 0.722R_1 - 1.432R_2 - 1.659R_3 + 2.599$$

$$\mathrm{MMb\%} = -0.159R_1 - 0.085R_2 + 1.262R_3 - 0.520$$

Where $R_1 = A_{582}/A_{525}$, $R_2 = A_{557}/A_{525}$, $R_3 = A_{503}/A_{525}$.

Total Meat Pigments A solvent extraction technique modified from Hornsey (1956) was used to determine the total meat pigments of chicken meat. Using the Ultrasonic probe sonicator (PCI Analytics, PKS-750F, Mumbai, India) 10 g of minced chicken meat sample was homogenized with 23 mL of a mixture containing 40 mL of acetone + 2 mL of distilled water and 1 mL of concentrated HCl. The remaining solution was added and kept for 1 hr with intermittent mixing. This solution was then filtered which gave a solution of acid hematin in 80% acetone. The filtrate was composed of hematin derived from any uncombined meat pigments present, together with that resulting from the oxidized pigments. At 640 nm, the optical density of the filtrate is measured. Distilled water was used as a blank. The obtained absorbance was then multiplied by a factor of 680 which gave the concentration of total pigments present in meat as ppm of hematin. Heme iron content was calculated as follows by using the hematin concentration.

$$\text{Haematin (ppm) (a)} = \text{Abs (640 nm)} \times 680$$

$$\text{Haeme iron} = (a \times 8.82)/100$$

CIELAB Color Space The instrumental color was measured using HunterLab Apparatus (MiniScan EZ 4500, HunterLab, VA). The chicken meat sample was placed below the aperture of the HunterLab Apparatus. The lightness (L*), redness (a*), and yellowness (b*) color units were recorded by comparing the meat sample with that of standard black and white plates. The color coordinates were measured 5 times in each meat sample.

Gas Concentration The concentrations of O_2, CO_2, and N_2 were measured by inserting the needle probe inside the packaging. The packaged meat samples were analyzed every day before the beginning of the sensory evaluation and meat quality parameters analysis, for the exact amount of gases infused through the Gas analyzer Checkmate 3, (Dansensor LE 316/2015, a Mocon company, Denmark). The packages containing the optimally infused gases were taken for further study. The needle was inserted at 5 different places and the values were noted.

Warner-Bratzler Shear Force The shear force test was performed according to Warner-Bratzler. Round cores of 1.27 cm (0.5 inches) in diameter were removed parallel to the longitudinal orientation of the muscle fibers so that the shearing action is perpendicular to the longitudinal orientation of the muscle fibers. The cores were obtained using a hand-held coring device. Then the cores were placed in the V- notched shear blade of the Texture analyzer (Tinius Olsen, HIKF, United Kingdom) and the cores were sheared with a crosshead speed of 200 mm/min. Each core was sheared once in the center. The peak shear force was recorded in newtons (N). Six cores were obtained from each chicken leg sample. So 6 shear force measurements were made for each meat treatment, that is, AP meat and MAP meat.

Microbiological Analysis

All the microbiological parameters of the meat sample were determined as per the methods described by APHA (2001). Readymade media from Hi-Media Laboratories Pvt Ltd., Mumbai were used for the enumeration of different microbes. Duplicate plates were prepared and the counts were expressed as $\log_{10}$ cfu/g.

Standard Plate Count About 23.5 g of Plate Count Agar (Hi-Media Laboratories Pvt Ltd., Mumbai Code No. M091) was suspended in 1,000 mL of distilled water. It was boiled to dissolve the media completely and sterilized in an autoclave at 15 lb pressure at 121°C for 15 min. The final pH of the media was adjusted to 7.0 ± 0.2. About 20 mL of the media was poured into sterile Petri plates and allowed to solidify under sterile conditions. Then the plates were kept in an incubator at 37 ± 1°C for 24 hr for a sterility check. After 24 hr 0.1mL of an aliquot from appropriate dilutions was poured onto the sterile Petri plates and spread on the plate with help of a sterile autoclavable spreader. Plates showing 30 to 300 colonies were counted. The number of colonies was multiplied with the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

Yeast and Mold Count About 39.0 grams of Potato Dextrose Agar (Hi-Media Laboratories Pvt Ltd., Mumbai Code No.M096) was suspended in 1,000 mL of distilled water. It was heated to boiling to dissolve the medium completely. Sterilized by, autoclaving at 15 lb pressure at 121°C for 15 min. It was cooled to 45 to 50° C. The medium was acidified with sterile 10% tartaric acid to adjust the pH to 3.5. The amount of acid required for 100 mL of sterile, cooled medium is approximately 1mL. The medium was mixed well and 20 mL was poured into sterile Petri plates. Then the plates were kept in an incubator at 37 ± 1°C for 24 h for a sterility check. After 24 hr 0.1 mL of an aliquot from appropriate dilutions was poured onto the sterile Petri plates and spread with help of a sterile autoclavable spreader. Plates showing 10 to 100 colonies were counted. The

number of colonies was multiplied with the reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

Sensory Evaluation

The sensory evaluation of the chicken meat samples was done as per the guidelines of the American Meat Science Association (AMSA, 2015). The quantitative descriptive analysis method was used to find the difference between the samples for various sensory attributes. The sensory quality of the chicken meat samples was judged based on the appearance, color, odor, and sliminess characteristics on a 5-point descriptive scale, 5 rated as extremely desirable and 1 rated as extremely undesirable. The samples were subjected to sensory evaluation by a sensory panel consisting of 7 members. A total of seven values were collected for each sample and the sensory evaluation was repeated thrice for all the treatments.

Statistical Analysis

The experiment has been repeated a minimum of 3 times in duplicate and the data obtained for different meat quality parameters were compiled and analyzed using SPSS (version 16.0 for Windows, SPSS, Chicago). The data were subjected to analysis of variance, (one-way ANOVA) for different groups and storage days. The least significant difference and Duncan's multiple range tests were applied for comparing the means to find the difference between groups and storage days. The color parameters were subjected to correlation analysis. The smallest difference (D_5%) between the 2 means was reported as significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Proximate composition

The mean values of the moisture, protein, and fat content of leg meat were 74.01%, 18.64%, and 4.65% respectively. Higher fat content may be due to the slaughter of older birds or birds fed on a high-fat diet as in intensive broiler farming. The meat from intensively reared broiler chickens was used in the current research. The fat content in the meat from younger birds remains high because of intensive feeding with high-fat feed formulation to achieve the slaughter weight in about one month. The differences in the proximate composition may be attributed to the fact that changes in the seasonal and nutrition status of birds, age of slaughter, and food composition. Clark et al. (1997) noticed that the moisture content of cooked chicken dark meat and light meat were 66% and 65% respectively. The moisture content of raw chicken breast and drumsticks were 76.5% and 75.5% respectively (Kongkachuichai et al., 2002). Cortez-Vega et al. (2012) indicated a mean value of moisture, protein, and crude fat percentage in the raw chicken breast as 75.82, 20.65, and 2.8 respectively. The total protein content of the chicken breast muscles was found to be 23.22% and 23.24% for outdoor and indoor rearing systems respectively (Michalczuk et al., 2014).

The results of moisture and protein% were similar to the mean values of the moisture, protein, and ether extract fat content of Cobb strain chicken breast showing 75.57%, 22.49%, and 0.75% respectively. Similarly, the mean values for thigh meat of Cobb strain for moisture, protein, and ether extract content were 76.14%, 19.86%, and 2.88% respectively (Souza et al., 2011).

pH

The pH of all the groups was significantly ($P < 0.05$) decreased, with storage time (Table 1). The results were supported by Vaithiyanathan et al. (2008) who expressed that the pH value of aerobic packaged spent hen leg meat at 4°C gradually decreased from 5.73 on day 0 to 5.30 on the 28th day of postmortem. The results differ from Stahlke et al. (2018) who reported that at all MAPs (MAP-1: Vacuum packaging, MAP-2: 69.6% N_2 + 30% CO_2 + 0.4%CO and MAP-3: 70%O_2 + 30%CO_2), the pH decreased with increasing slaughter age of lambs and increased with the longer storage period at 4°C for 35 d. Rapid pH decline in muscle may be related to the denaturation of myofibrillar and

Table 1. pH and TBARS changes in aerobic and modified atmosphere packaged chicken leg meat during refrigeration storage (4 ± 1°C).

Parameters/Groups	Days							
pH	0	3	6	9	12	15	18	21
AP-CLM	6.25 ± 0.05^{aA}	6.16 ± 0.04^{aA}	6.16 ± 0.02^{aA}	5.80 ± 0.05^{bB}	NA	NA	NA	NA
MAP-CLM20	6.40 ± 0.04^{aA}	6.25 ± 0.11^{aAB}	6.22 ± 0.04^{aAB}	6.16 ± 0.05^{aAB}	6.18 ± 0.04^{aAB}	6.14 ± 0.10^{aB}	6.12 ± 0.08^{aB}	6.05 ± 0.12^{aB}
MAP-CLM10	6.36 ± 0.07^{aA}	6.29 ± 0.12^{aA}	6.28 ± 0.08^{aAB}	6.19 ± 0.06^{aAB}	6.13 ± 0.08^{aAB}	6.11 ± 0.07^{aAB}	6.12 ± 0.04^{aAB}	6.03 ± 0.09^{aB}
MAP-CLM0	6.32 ± 0.03^{aA}	6.27 ± 0.08^{aAB}	6.22 ± 0.09^{aABC}	6.11 ± 0.08^{aABC}	6.11 ± 0.08^{aABC}	6.04 ± 0.04^{aBC}	6.04 ± 0.04^{aC}	6.02 ± 0.10^{aC}
TBARS (mg/kg)								
AP-CLM	0.08 ± 0.01^{aD}	0.11 ± 0.01^{aC}	0.12 ± 0.01^{aB}	0.14 ± 0.01^{aA}	NA	NA	NA	NA
MAP-CLM20	0.07 ± 0.01^{aD}	0.08 ± 0.01^{bCD}	0.08 ± 0.01^{bBCD}	0.09 ± 0.01^{bABC}	0.09 ± 0.01^{aABC}	0.09 ± 0.01^{aAB}	0.09 ± 0.01^{aAB}	0.1 ± 0.01^{aA}
MAP-CLM10	0.07 ± 0.01^{aE}	0.07 ± 0.01^{bDE}	0.08 ± 0.01^{cDE}	0.08 ± 0.01^{bCD}	0.09 ± 0.01^{aBC}	0.09 ± 0.01^{aAB}	0.09 ± 0.01^{aAB}	0.09 ± 0.01^{aA}
MAP-CLM0	0.08 ± 0.01^{aC}	0.08 ± 0.01^{bBC}	0.08 ± 0.01^{bBC}	0.08 ± 0.01^{bBC}	0.09 ± 0.01^{aBC}	0.09 ± 0.01^{aB}	0.09 ± 0.01^{aB}	0.11 ± 0.01^{aA}

n = 6; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM = Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat (20%O_2 + 30% CO_2 + 50%N_2); MAP-CLM10 = MAP leg meat (10%O_2 + 40% CO_2 + 50%N_2); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed.

sarcoplasmic proteins, increased actomyosin contractions, and the change in the meat structure (Yu et al., 2005). The pH of the aerobic packaged chicken leg meat (**AP-CLM**) group was significantly ($P < 0.05$) lower than modified atmosphere packaged chicken meat (**MAP-CLM**) groups on day 9 of the refrigerated storage period. According to Milijasevic et al. (2019), the development of lactic acid bacteria is the major cause of the reduction in pH of packaged fish. Whereas the lactic acid formation due to postmortem glycolysis of meat is the cause of the decrease in pH during prolonged storage. The higher pH values at the initial days of storage in meat are due to the meat maturation process that involves myofibrillar structure degradation by enzymes (Rodrigues et al., 2018).

There was no significant difference ($P > 0.05$) in the pH values within MAP-CLM groups during the whole storage period. Ariff et al. (2011) expressed that the pH decrease was because of the reaction between carbon dioxide and water, which resulted in the formation of carbonic acid during the first 2 wk of storage.

Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS values of both aerobic packaged and modified atmosphere packaged chicken leg meat were significantly ($P < 0.05$) increasing with storage time (Table 1). The increasing trend of TBARS value is due to increased oxidation of unsaturated fatty acids during storage which is accelerated in the presence of oxygen (Mendes et al., 2008). Tomankova et al. (2012) observed that a significant increase in the TBARS value of chicken hindquarters occurred during storage time. This increase was more pronounced in oxygen MA than in the argon MA packaging. Muhlisin et al. (2014) pointed the TBARS of *longissimus dorsi* of Korean native pigs in MAP-3 (70% O_2 + 20%CO_2 + 10%N_2) was higher than that of MAP-2 (30%O_2 + 20%CO_2 + 50%N_2) and the TBARS value of MAP-2 was higher than that of MAP-1 (0%O_2 + 20%CO_2 + 80%N_2) and VP.

The TBARS values of the AP-CLM group were significantly ($P < 0.05$) lower than MAP-CLM groups on days 3, 6, and 9 of the refrigerated storage period. There was no significant difference ($P > 0.05$) in the TBARS values within MAP-CLM groups during the whole storage period except for MAP-CLM10, which showed a significantly ($P < 0.05$) lower value on day 6. Malondialdehyde and other products of lipid oxidation are not stable and are decomposed into organic forms, which are not detected by the TBARS test (Maqsood and Benjakul, 2010). Initial TBARS values of beef steaks stored at 4 ± 1°C for 35 d were determined as 0.142, 0.144, and 0.183 mg MDA/kg for control (air), MAP-1 (60%O_2 + 40%CO_2), and MAP-2 (60%O_2 + 20%CO_2 + 20%N_2) samples, respectively. At the end of the storage period, TBARS values increased to 0.810, 0.680, and 0.689 MDA/kg, respectively (Bagdatli and Kayaardi, 2015).

Myoglobin Content

The deoxymyoglobin contents of aerobic packaged and modified atmosphere packaged chicken leg meat increased significantly ($P < 0.05$) with storage time (Table 2). Muscles contain metmyoglobin reducing enzymes, which catalyze the reduction of MMb to DMb which then reacts with oxygen to form OMb (Leygonie et al., 2012). The deoxymyoglobin content of the AP-CLM group was significantly ($P < 0.05$) higher compared to MAP-CLM groups on days 0, 3, and 9 of the refrigerated storage period. There was no significant difference ($P > 0.05$) in DMb between the MAP-CLM groups during the whole storage time.

The metmyoglobin content of the AP-CLM and MAP-CLM10 significantly ($P < 0.05$) increased with storage time (Table 2). There was no significant difference ($P > 0.05$) in MAP-CLM20 and MAP-CLM0 groups with the storage period. The metmyoglobin content of the AP-CLM group was significantly ($P < 0.05$) lower compared to MAP-CLM groups on days 0 and 3 of the refrigerated storage period. The MMb content of MAP-CLM20 was significantly ($P < 0.05$) lower on days 18 and 21 and significantly ($P < 0.05$) higher on days 6. The metmyoglobin content of normal pH beef steak increased from 4.31% to 31.6% as storage time extended, with distinguishable differences between normal pH and dark cutting groups after 4 days of storage. Steaks in 20% O_2 − MAP showed the highest metmyoglobin content after day 7, explained by the relatively low O_2 partial pressure on the meat surface, enhancing metmyoglobin formation (Lu et al., 2020).

The oxymyoglobin content of all the groups was found to be significantly ($P < 0.05$) decreasing with storage time (Table 2), which may be due to a decrease in oxygen% with storage time. There was no significant difference ($P > 0.05$) in OMb between the AP-CLM group and MAP-CLM groups during the whole storage time. The OMb content of the MAP-CLM20 was significantly ($P < 0.05$) higher compared to other MAP groups. Results were similar to Teuteberg et al. (2021) who found that on day 1, pork samples frozen for 12 wk (−18°C and −80°C) showed, independent of the storage temperature, significantly lower deoxymyoglobin and metmyoglobin and high oxymyoglobin % in comparison to samples, frozen for 24 wk (−18°C and −80°C). Teuteberg et al. (2021) explained that the increased MMb% and decreased OMb% in the pork samples, previously frozen and stored for 24 wk (−18°C and −80°C) was due to a decrease or loss of myoglobin reducing activity during freezing.

Total Meat Pigments (TMP)

The total meat pigments concentration and heme iron content of all the groups were significantly ($P < 0.05$) decreased, with storage time (Table 3). The TMP concentration and heme iron content of the AP-CLM group were significantly ($P < 0.05$) higher compared to MAP-CLM groups on days 3 and 6 of the refrigerated storage

Table 2. Myoglobin form changes in aerobic and modified atmospheric packaged chicken leg meat during refrigeration storage (4 ± 1°C).

Parameters/Groups	Days							
Deoxymyoglobin(%)	0	3	6	9	12	15	18	21
AP-CLM	26.45 ± 0.73^{aAB}	25.69 ± 0.58^{aB}	25.40 ± 0.26^{aB}	27.25 ± 0.31^{aA}	NA	NA	NA	NA
MAP-CLM20	22.02 ± 1.44^{bB}	23.44 ± 0.54^{bAB}	23.74 ± 1.08^{aAB}	25.14 ± 0.71^{bAB}	24.54 ± 1.37^{aAB}	24.44 ± 1.00^{aAB}	25.50 ± 0.48^{aA}	26.00 ± 1.26^{aA}
MAP-CLM10	22.19 ± 0.29^{bC}	24.47 ± 0.64^{abBC}	23.77 ± 0.89^{aBC}	24.82 ± 0.54^{bBC}	24.40 ± 0.52^{aBC}	25.20 ± 0.33^{aBC}	26.72 ± 1.58^{aAB}	28.75 ± 2.35^{aA}
MAP-CLM0	21.19 ± 1.68^{bC}	23.50 ± 0.69^{bBC}	24.27 ± 0.46^{aBC}	24.04 ± 0.76^{bBC}	23.67 ± 1.11^{aBC}	25.07 ± 0.42^{aAB}	25.67 ± 0.82^{aAB}	28.32 ± 1.98^{aA}
Metmyoglobin (%)								
AP-CLM	55.99 ± 1.33^{bB}	56.60 ± 0.52^{bB}	59.97 ± 0.24^{aA}	59.77 ± 0.11^{aA}	NA	NA	NA	NA
MAP-CLM20	58.67 ± 1.20^{abA}	60.65 ± 1.27^{aA}	59.89 ± 2.26^{aA}	60.17 ± 0.41^{aA}	59.70 ± 0.66^{aA}	60.51 ± 0.79^{aA}	60.51 ± 1.97^{bA}	59.27 ± 1.59^{bA}
MAP-CLM10	59.62 ± 0.36^{aC}	58.90 ± 0.83^{abC}	60.77 ± 0.65^{aBC}	60.80 ± 1.24^{aBC}	61.48 ± 1.35^{aBC}	62.30 ± 0.71^{aABC}	64.30 ± 0.51^{aAB}	65.70 ± 2.51^{aA}
MAP-CLM0	59.79 ± 1.18^{aA}	60.02 ± 0.97^{bA}	60.19 ± 0.67^{aA}	60.90 ± 0.62^{aA}	62.17 ± 1.05^{aA}	61.71 ± 1.10^{aA}	62.04 ± 0.89^{abA}	62.50 ± 0.64^{abA}
Oxymyoglobin (%)								
AP-CLM	17.79 ± 0.99^{aA}	16.40 ± 0.75^{abB}	14.79 ± 0.41^{aC}	14.54 ± 0.33^{aC}	NA	NA	NA	NA
MAP-CLM20	19.55 ± 2.00^{aA}	17.75 ± 0.72^{aAB}	16.64 ± 2.00^{aABC}	14.75 ± 0.82^{aBC}	14.18 ± 0.23^{aBC}	14.87 ± 0.67^{aBC}	13.64 ± 1.40^{aBC}	13.47 ± 1.28^{aC}
MAP-CLM10	17.72 ± 1.86^{aA}	15.09 ± 0.77^{bAB}	14.43 ± 0.64^{aAB}	14.65 ± 0.92^{aAB}	14.37 ± 1.49^{aAB}	14.47 ± 2.10^{abAB}	10.67 ± 0.46^{aBC}	6.50 ± 4.25^{abC}
MAP-CLM0	18.87 ± 0.72^{aA}	16.67 ± 0.63^{abAB}	16.84 ± 1.53^{aAB}	14.43 ± 0.61^{aBC}	12.60 ± 0.75^{aCD}	11.37 ± 0.36^{bCD}	11.77 ± 1.91^{aCD}	10.94 ± 1.06^{aD}

n = 6; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM = Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat (20%O_2 + 30% CO_2 + 50%N_2); MAP-CLM10 = MAP leg meat (10%O_2 + 40% CO_2 + 50%N_2); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed.

period. The TMP concentration and heme iron content of MAP-CLM20 were significantly ($P < 0.05$) lower on days 3, 6, and 9 and significantly ($P < 0.05$) higher on days 18 and 21 compared to other MAP groups. Cooked patties (core temperature 71°C on a gas grill of 176°C) composed of ground chuck with pH 6.0 exhibited a more intense stable pink color than patties with a pH of 5.7 (Mendenhall, 1989). Clark et al. (1997) expressed that the heme iron values of cooked chicken dark meat and light meat were 5.6 μg/g and 2.3 μg/g. Valenzuela et al. (2009) noticed that the mean values of heme iron in loin and brisket of beef were 0.9 mg/100 g and 0.8 mg/100 g respectively.

The total meat pigments concentration gradually decreased in the meat up to day 11 of storage, by 36.23% for goose meat packed in MAP ($80\%O_2 + 20\%CO_2$) and 23.77% for meat packed in vacuum. After 24 h, the concentration of total meat pigments reached 2.65 mg/g; however, on day 11 of storage, it reached 1.69 mg/g for MAP and 2.02 mg/g for vacuum packaged goose meat (Orkusz et al., 2017). The trends in the changes of pigments in chicken meat found in the current research are similar to the results of Orkusz et al. (2017).

CIELAB Color Space

The lightness (L*) value increased significantly ($P < 0.05$) in the AP-CLM during the storage period (Table 4). But there was no significant difference ($P > 0.05$) in L* values between the AP-CLM and MAP-CLM during the prolonged storage. An increase in the lightness value may be due to structural variations such as protein oxidation or cross-linking, most likely under highly oxidized conditions (Lu et al., 2020). Guo et al. (2018) observed that the initial L* value of samples was 37.78 and the values increased significantly only on day 4 and then remained to be stable.

The redness (a*) values of the AP-CLM was significantly ($P < 0.05$) higher than MAP-CLM on days 0 and 9 of the refrigerated storage. The redness (a*) value decreased significantly ($P < 0.05$) in MAP-CLM10 on day 15. MAP-CLM20 showed a significantly ($P < 0.05$) higher redness (a*) value with storage period (Table 4), which may be due to more oxygen concentration. The color of muscle is affected by numerous factors the most important of which are sex, age, intramuscular fat, moisture percentage, preslaughtering conditions, processing methods, and presence of muscle pigments (Mothershaw et al., 2009). According to Li et al. (2020), the older chicken muscles exhibited darker, redder, and less yellow color than the chicken muscles with younger age. The decreased a* values are usually related to the gradual formation of metmyoglobin which causes meat discoloration (Insausti et al., 2001). In the present research, the decreased redness values corroborated with the increase in metmyoglobin% of chicken leg meat during prolonged storage.

There was no significant ($P > 0.05$) difference in yellowness (b*) values between AP-CLM and MAP-CLM

Table 3. Total meat pigments and Heame iron changes in aerobic and modified atmosphere packaged chicken leg meat during refrigeration storage (4 ± 1°C).

Parameters/ Groups	Days							
Total meat pigments (ppm)	0	3	6	9	12	15	18	21
AP-CLM	7.41 ± 0.30^{aA}	6.97 ± 0.15^{aA}	5.60 ± 0.23^{aB}	4.01 ± 0.31^{abC}	NA	NA	NA	NA
MAP-CLM20	6.04 ± 1.26^{aA}	3.78 ± 0.25^{bB}	3.60 ± 0.14^{bB}	3.44 ± 0.12^{bB}	3.88 ± 0.24^{aB}	4.15 ± 0.39^{aB}	4.82 ± 0.43^{aAB}	4.94 ± 0.55^{aAB}
MAP-CLM10	6.51 ± 0.25^{aA}	6.34 ± 1.16^{aA}	5.92 ± 0.41^{aA}	4.29 ± 0.37^{aB}	4.01 ± 0.29^{aB}	3.89 ± 0.39^{aB}	3.66 ± 0.33^{bB}	3.70 ± 0.29^{bB}
MAP-CLM0	6.85 ± 1.39^{aA}	5.76 ± 0.19^{aAB}	5.28 ± 0.09^{aABC}	4.74 ± 0.19^{aBCD}	4.06 ± 0.33^{aBCD}	4.12 ± 0.44^{aBCD}	3.84 ± 0.36^{bCD}	3.44 ± 0.24^{bD}
Heme iron (mg/kg)								
AP-CLM	6.53 ± 0.27^{aA}	6.15 ± 0.13^{aA}	4.93 ± 0.20^{aB}	3.61 ± 0.27^{abC}	NA	NA	NA	NA
MAP-CLM20	5.32 ± 1.11^{aA}	3.33 ± 0.22^{bB}	3.17 ± 0.12^{bB}	3.02 ± 0.10^{bB}	3.41 ± 0.22^{aB}	3.63 ± 0.34^{aB}	4.25 ± 0.38^{aAB}	4.35 ± 0.48^{aAB}
MAP-CLM10	5.73 ± 0.22^{aA}	5.58 ± 1.02^{aA}	5.21 ± 0.36^{aA}	3.77 ± 0.33^{aB}	3.53 ± 0.26^{aB}	3.42 ± 0.35^{aB}	3.22 ± 0.29^{bB}	3.26 ± 0.26^{bB}
MAP-CLM0	6.03 ± 1.23^{aA}	5.07 ± 0.17^{aAB}	4.99 ± 0.15^{aAB}	4.17 ± 0.17^{aBC}	3.57 ± 0.30^{aBC}	3.65 ± 0.39^{aBC}	3.38 ± 0.32^{bC}	3.03 ± 0.22^{bC}

n = 6; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM= Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat ($20\%O_2$ + 30% CO_2 + $50\%N_2$); MAP-CLM10 = MAP leg meat ($10\%O_2$ + 40% CO_2 + $50\%N_2$); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed.

during the storage period (Table 4). The yellowness (b*) value was significantly ($P < 0.05$) higher in MAP-CLM0 on day 15. Orkusz et al. (2017) found that goose meat packaged in MAP ($80\%O_2$ + $20\%CO_2$) for 7 d showed an increase in yellowness value at 4°C. The change in b* value is attributed to the significant decrease in total meat pigments and oxymyoglobin% with a relative increase in metmyogloibin%.

Gas Concentration

The oxygen% of all the groups was significantly ($P < 0.05$) decreased, with storage time (Table 5), which might be because of the consumption of oxygen by putrefactive bacteria and the permeability of packaging material. Results were similar to Chemiel et al. (2018) who noticed that the content of oxygen decreased with storage time in chicken breast meat packages stored in the cooling room (2 ± 0.5°C) as well as in refrigerated display case (<4°C). The lowest O_2 content was observed in MAP (75% O_2 + 25% CO_2) packages stored for 9 d in the display case. The carbon dioxide% of all the groups was significantly ($P < 0.05$) decreased, with storage time (Table 5), maybe due to permeation or biochemical conversion through respiratory activity or dissolution in the aqueous phase of meat. According to Abdullah et al. (2017), a reduction in CO_2 content in MAP is a result of its conversion to carbonic acid. The results were similar to Jimenez et al. (1997) who noticed that the concentration of the CO_2 decreased with increasing storage time (21 d) at 4°C in both MAPs ($70\%N_2$ + $30\%CO_2$ and $30\%N_2$ + $70\%CO_2$) packaged chicken breasts. The nitrogen% of all the groups significantly ($P < 0.05$) increasing, with storage time (Table 5). The O_2%, CO_2%, and N_2% of the aerobic and modified atmosphere packaged chicken meat differed significantly ($P < 0.05$) during the refrigerated storage period.

In MAP-CLM20, the O_2 and CO_2 decreased by nearly 8 and 11% respectively and N_2 increased by nearly 19%. In MAP-CLM10, the O_2 and CO_2 decreased by nearly 4

Table 4. CIELAB color space changes in aerobic and modified atmosphere packaged chicken leg meat during refrigeration storage (4 ± 1°C).

Parameters/ Groups	Days							
L* value	0	3	6	9	12	15	18	21
AP-CLM	63.67 ± 4.19^{aB}	65.17 ± 3.44^{aB}	67.67 ± 3.26^{aAB}	73.34 ± 3.11^{aA}	NA	NA	NA	NA
MAP-CLM20	68.50 ± 3.34^{aA}	70.84 ± 3.42^{aA}	76.34 ± 5.08^{aA}	75.34 ± 0.67^{aA}	72.00 ± 2.53^{aA}	69.17 ± 4.17^{aA}	63.67 ± 3.91^{aA}	72.00 ± 6.67^{aA}
MAP-CLM10	67.34 ± 3.47^{aA}	71.00 ± 1.57^{aA}	71.84 ± 1.82^{aA}	71.67 ± 3.41^{aA}	71.00 ± 2.52^{aA}	69.67 ± 8.35^{aA}	64.00 ± 6.56^{aA}	70.50 ± 5.05^{aA}
MAP-CLM0	69.17 ± 1.85^{aA}	68.50 ± 4.94^{aA}	66.67 ± 7.17^{aA}	74.00 ± 0.45^{aA}	67.00 ± 4.78^{aA}	65.34 ± 4.75^{aA}	70.17 ± 4.60^{aA}	72.17 ± 3.68^{aA}
a* value								
AP-CLM	23.84 ± 3.82^{aA}	20.17 ± 2.94^{aA}	22.84 ± 2.80^{aA}	19.17 ± 2.10^{aA}	NA	NA	NA	NA
MAP-CLM20	13.00 ± 2.58^{bC}	13.17 ± 0.91^{aC}	15.17 ± 1.22^{aBC}	15.83 ± 2.96^{abBC}	16.67 ± 1.23^{aBC}	20.17 ± 2.09^{abABC}	21.83 ± 3.47^{aAB}	26.5 ± 4.12^{aA}
MAP-CLM10	22.34 ± 3.95^{abA}	19.50 ± 2.66^{aA}	19.50 ± 2.60^{aA}	14.00 ± 1.90^{abA}	15.84 ± 0.91^{aA}	14.00 ± 2.78^{bA}	22.67 ± 4.77^{aA}	19.67 ± 2.88^{aA}
MAP-CLM0	22.50 ± 1.41^{abA}	20.50 ± 3.49^{aA}	20.67 ± 5.34^{aA}	$12.00 \pm 0..77^{bA}$	16.34 ± 1.48^{aA}	23.67 ± 4.64^{aA}	24.17 ± 5.28^{aA}	20.84 ± 4.21^{aA}
b* value								
AP-CLM	29.17 ± 5.70^{aA}	32.84 ± 4.23^{aA}	32.50 ± 4.10^{aA}	31.50 ± 2.80^{aA}	NA	NA	NA	NA
MAP-CLM20	38.34 ± 2.59^{aA}	24.34 ± 4.51^{aA}	28.00 ± 3.96^{aA}	26.83 ± 5.28^{aA}	37.00 ± 3.70^{aA}	26.00 ± 3.91^{bA}	36.00 ± 8.56^{aA}	29.84 ± 5.67^{aA}
MAP-CLM10	27.17 ± 4.77^{aA}	27.00 ± 2.78^{aA}	29.50 ± 5.52^{aA}	32.34 ± 4.39^{aA}	28.00 ± 4.62^{aA}	25.67 ± 4.01^{bA}	35.34 ± 5.59^{aA}	23.50 ± 4.01^{aA}
MAP-CLM0	32.00 ± 1.63^{aA}	30.17 ± 5.09^{aA}	29.84 ± 5.18^{aA}	30.50 ± 2.87^{aA}	31.67 ± 5.89^{aA}	39.19 ± 4.52^{aA}	34.67 ± 6.14^{aA}	34.17 ± 6.26^{aA}

n = 6; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM = Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat ($20\%O_2$+30% CO_2+$50\%N_2$); MAP-CLM10 = MAP leg meat ($10\%O_2$ + 40% CO_2 + $50\%N_2$); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed.

Table 5. Gas concentration changes in aerobic and modified atmosphere packaged chicken leg meat during refrigeration storage (4 ± 1° C).

Parameters/ Groups	Days							
Oxygen (%)	0	3	6	9	12	15	18	21
AP-CLM	21.44 ± 0.06^{aA}	19.97 ± 0.30^{aB}	18.84 ± 0.46^{aC}	17.37 ± 0.66^{aD}	NA	NA	NA	NA
MAP-CLM20	19.19 ± 1.14^{bA}	17.87 ± 1.25^{aAB}	16.05 ± 1.32^{bABC}	15.55 ± 0.94^{aCD}	15.29 ± 1.79^{aCD}	14.3 ± 0.59^{aCD}	13.55 ± 0.81^{aD}	12.72 ± 0.84^{aD}
MAP-CLM10	9.16 ± 0.57^{cA}	7.98 ± 1.03^{bAB}	6.63 ± 0.33^{cB}	6.78 ± 0.96^{bB}	6.8 ± 0.32^{bB}	6.8 ± 0.39^{bB}	6.84 ± 0.66^{bB}	6.17 ± 0.92^{bB}
MAP-CLM0	1.79 ± 0.16^{dA}	1.53 ± 0.30^{cA}	1.33 ± 0.44^{dAB}	1.54 ± 0.35^{cA}	1.95 ± 0.58^{cA}	1.33 ± 0.36^{cA}	0.38 ± 0.15^{cBC}	0.03 ± 0.01^{cC}
Carbon dioxide (%)								
AP-CLM	1.90 ± 0.13^{dA}	1.60 ± 0.27^{cB}	1.50 ± 0.13^{dB}	0.27 ± 0.02^{cC}	NA	NA	NA	NA
MAP-CLM20	31.32 ± 0.66^{bA}	30.87 ± 1.14^{aA}	29.82 ± 1.38^{bAB}	29.39 ± 1.67^{aAB}	28.44 ± 2.29^{aABC}	26.82 ± 1.93^{bABC}	19.47 ± 6.11^{bBC}	18.49 ± 5.81^{bC}
MAP-CLM10	38.9 ± 0.86^{aA}	33.32 ± 1.65^{aB}	33.24 ± 1.54^{aB}	31.72 ± 0.61^{aB}	31.77 ± 3.05^{aB}	31.25 ± 0.89^{aB}	30.85 ± 1.52^{aB}	30.70 ± 0.82^{aB}
MAP-CLM0	20.54 ± 0.21^{cA}	20.54 ± 0.09^{bA}	20.37 ± 0.08^{cA}	19.67 ± 0.06^{bB}	19.24 ± 0.15^{bC}	19.00 ± 0.22^{cCD}	18.70 ± 0.73^{bDE}	18.33 ± 0.20^{bE}
Nitrogen (%)								
AP-CLM	76.67 ± 0.18^{aD}	78.43 ± 0.36^{aC}	79.67 ± 0.37^{aB}	82.37 ± 0.67^{aA}	NA	NA	NA	NA
MAP-CLM20	49.7 ± 1.70^{bC}	51.27 ± 0.67^{cC}	54.13 ± 0.31^{cC}	55.07 ± 2.60^{cC}	56.61 ± 1.08^{cBC}	58.88 ± 1.92^{cABC}	66.98 ± 6.80^{bAB}	68.80 ± 6.33^{bA}
MAP-CLM10	51.94 ± 1.35^{bB}	58.71 ± 1.82^{bA}	60.14 ± 1.60^{bA}	61.51 ± 0.57^{bA}	61.44 ± 2.77^{bA}	61.96 ± 0.54^{bA}	62.32 ± 0.91^{bA}	63.13 ± 0.78^{bA}
MAP-CLM0	77.68 ± 0.28^{aD}	77.94 ± 0.32^{aCD}	78.30 ± 0.37^{aCD}	78.80 ± 0.30^{aBC}	78.83 ± 0.69^{aBC}	79.67 ± 0.33^{aB}	80.92 ± 0.14^{aA}	81.64 ± 0.30^{aA}

n = 6; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM= Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat (20%O_2 + 30% CO_2 + 50%N_2); MAP-CLM10 = MAP leg meat (10%O_2 + 40% CO_2 + 50%N_2); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed.

and 10% respectively and N_2 increased by nearly 14%. In MAP-CLM0 CO_2 decreased nearly by 2% and N_2 increased by nearly 2%.

In MAP (80%O_2 + 13%CO_2 + 7%N_2) packaged chicken breast meat, the percent of oxygen decreased by nearly 10%, carbon dioxide increased by more than 2.5%, and the concentration of nitrogen increased more than twice after 7 d of storage at 2°C (Kot vel Lawecka et al., 2019).

Warner-Bratzler Shear Force (WBSF)

The shear force values of aerobic packaged and modified atmosphere packaged chicken leg meat increased significantly ($P < 0.05$) with the storage period. The increase in WBSF may be due to muscle fiber shrinkage due to loss of water during thawing which increases the toughness of muscles. The WBSF value of the AP-CLM group was significantly ($P < 0.05$) lower than MAP-CLM groups on days 0, 3, and, 6 of the refrigerated storage period (Table 6). The WBSF value was significantly ($P < 0.05$) lower in MAP-CLM20 on day 3 of the refrigerated storage period. Yu et al. (2005) observed no significant difference in shear value between chicken breast and leg muscles.

Mbaga et al. (2014) observed that all the chicken meat cuts showed a prominent decline in the shear force values during the first 6 hr of aging, then the decline was

Table 6. Warner-Bratzler shear force value, standard plate count, and yeast and mold count changes in aerobic and modified atmosphere packaged chicken leg meat during refrigeration storage (4 ± 1°C).

Parameters/ Groups	Days							
Warner-Bratzler shear force value (N)	0	3	6	9	12	15	18	21
AP-CLM	4.12 ± 0.48^{bC}	6.57 ± 0.27^{cB}	5.89 ± 0.83^{bB}	9.12 ± 0.21^{aA}	NA	NA	NA	NA
MAP-CLM20	6.43 ± 0.21^{aD}	7.69 ± 0.19^{bcCD}	8.59 ± 0.52^{abBCD}	8.66 ± 0.72^{aBCD}	9.01 ± 0.09^{aBCD}	10.09 ± 0.44^{aBC}	10.53 ± 0.69^{aAB}	12.92 ± 2.12^{abA}
MAP-CLM10	6.89 ± 0.20^{aB}	9.46 ± 0.56^{aB}	9.85 ± 1.41^{aB}	7.86 ± 0.27^{aB}	8.78 ± 0.19^{aB}	9.50 ± 0.62^{aB}	9.20 ± 0.65^{aB}	18.08 ± 5.81^{aA}
MAP-CLM0	5.98 ± 0.24^{aB}	8.40 ± 0.47^{abB}	7.82 ± 0.58^{abB}	8.35 ± 0.08^{aB}	8.75 ± 0.18^{aB}	11.05 ± 1.32^{aB}	10.55 ± 2.31^{aB}	18.57 ± 5.92^{aA}
Standard plate count (log_{10} cfu/g)								
AP-CLM	4.18 ± 0.08^{bD}	5.02 ± 0.02^{bC}	5.25 ± 0.02^{bB}	7.41 ± 0.01^{aA}	NA	NA	NA	NA
MAP-CLM20	5.71 ± 0.20^{aC}	6.24 ± 0.35^{aBC}	6.50 ± 0.23^{aBC}	6.90 ± 0.17^{bB}	6.97 ± 0.41^{aB}	6.90 ± 0.20^{aB}	8.41 ± 0.31^{aA}	8.43 ± 0.32^{aA}
MAP-CLM10	5.76 ± 0.22^{aC}	6.18 ± 0.39^{aBC}	6.32 ± 0.37^{aBC}	6.54 ± 0.15^{bBC}	6.22 ± 0.33^{aBC}	6.89 ± 0.19^{aB}	8.13 ± 0.37^{aA}	8.42 ± 0.32^{aA}
MAP-CLM0	5.77 ± 0.17^{aC}	6.23 ± 0.36^{aBC}	6.43 ± 0.31^{aBC}	6.62 ± 0.18^{bBC}	6.90 ± 0.44^{aB}	6.90 ± 0.17^{aB}	8.16 ± 0.38^{aA}	8.43 ± 0.32^{aA}
Yeast and mold count (log_{10} cfu/g)								
AP-CLM	1.11 ± 0.71^{aB}	1.31 ± 0.83^{aB}	1.46 ± 0.92^{aB}	3.57 ± 1.17^{aA}	NA	NA	NA	NA
MAP-CLM20	1.43 ± 0.91^{aA}	1.56 ± 0.99^{aA}	1.49 ± 0.94^{aA}	1.53 ± 0.97^{aA}	1.90 ± 1.20^{aA}	1.90 ± 1.20^{aA}	3.36 ± 1.08^{aA}	4.17 ± 0.14^{aA}
MAP-CLM10	1.43 ± 0.91^{aA}	1.43 ± 0.91^{aA}	1.43 ± 0.91^{aA}	1.43 ± 0.91^{aA}	1.92 ± 1.22^{aA}	1.82 ± 1.15^{aA}	3.31 ± 1.06^{aA}	3.39 ± 1.09^{aA}
MAP-CLM0	1.49 ± 0.94^{aA}	1.62 ± 1.02^{aA}	1.61 ± 1.02^{aA}	1.49 ± 0.94^{aA}	1.82 ± 1.15^{aA}	1.82 ± 1.15^{aA}	3.26 ± 1.04^{aA}	3.46 ± 1.12^{aA}

n = 6; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM = Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat (20%O_2 + 30% CO_2 + 50%N_2); MAP-CLM10 = MAP leg meat (10%O_2 + 40% CO_2 + 50%N_2); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed.

gradual with less than 13.3 N for drumstick and 18.9 N for both thigh and breast within 12 hr of post mortem. No significant variations were detected in WBSF among different MAPs (MAP-1: Vacuum, MAP-2: 69.6%N_2 + 30%CO_2 + 0.4%CO and MAP-3: 70%O_2 + 30%CO_2) or Slaughter age (4 and 8 mo) treatments ($P > 0.05$) after 35 d of storage of *longissimus thoracis et lumborum* of lamb at 4°C. However, WBSF was affected by storage time (Stahlke et al., 2018).

Kim et al. (2012) indicated decreased shear force values during the storage period, which was attributed to the action of proteolytic enzymes on muscle myofibrils during an extended storage period of 9 wk at −1.5°C. The present research showed a different finding that the shear force values increased significantly during the prolonged storage period at 4 ± °C. The difference in observation might be due to the difference in storage temperature as freezing tenderizes the meat leading to a decrease in shear force as in the previous case. According to Chen et al. (2007), probably the larger diameter of muscle fibers resulted in higher shear force, partly due to the greater thickness of the perimysium of the muscles.

Microbiological Analysis

Standard Plate Count (SPC) The standard plate count of aerobic packaged and modified atmosphere packaged chicken leg meat increased significantly ($P < 0.05$) during the storage period (Table 6). The results were similar to Chemiel et al. (2018) who reported that the total plate count (**TPC**) systematically increased with the storage time of breast meat in MAP (75% O_2 + 25% CO_2) packages at 4°C for 9 d. The first indication of spoilage in fresh chicken meat is the production of off-odors, which turn out to be obvious when bacterial numbers reach around 10^7 cfu/cm^2 (Obrein et al., 1995). At this point, the microorganisms have exhausted levels of glucose and amino acids in the meat as a growth substrate. In addition to the gaseous environment, which influences microorganisms, the durability of meat in MAP depends on extrinsic and intrinsic factors such as meat pH, initial microbial load, the temperature during storage, and packaging procedures. Thus, the mutual effects of high pH and high microbial numbers will restrict the shelf-life of the meat (Rodriguez-Calleja et al., 2010). Kandeepan and Biswas (2005) reported that the TPC of buffalo meat increased during chiller storage but it decreased during freezer storage.

The SPC of the AP-CLM group was significantly ($P < 0.05$) higher than MAP-CLM groups on day 9 and significantly ($P < 0.05$) lower on days 0, 3, and 6 of the refrigerated storage period. Total viable count levels for all beef steaks of different pH (normal: 5.40−5.79; intermediate: 5.80−6.09; and high: ≥6.10) were found to increase with storage time of 14 d at 2°C throughout the experiment in both high-oxygen MAP (80%O_2 + 20%CO_2) and carbon monoxide−MAP (Yang et al., 2021). Microbial spoilage is of great concern for poultry meat as it has a limited storage time under refrigerated conditions of 0 to 10°C (Chouliara et al., 2008). As per Food Safety and Standards Regulations 2011, relating to microbiological requirements of food products, the Indian Standards IS:5402 states that the maximum permissible limit for total plate count in chilled meat is 10^5 cfu/g. The chilled meat should be rejected as the total plate count reaches/exceeds the level of 5×10^6 cfu/g (FSSAI, 2010). There was no significant difference ($P > 0.05$) in the SPC values within MAP-CLM groups and during the whole storage period. At 3 d of storage, total aerobic plate counts of MAP-2 (30%O_2 + 20%CO_2 + 50%N_2) and MAP-3 (70%O_2 + 20%CO_2 + 10%N_2) were higher than those of VP and MAP-1 (0%O_2 + 20%CO_2 + 80%N_2) for *longissimus dorsi* of Korean native pig. At 6 d of storage, total aerobic plate counts of MAP-3 were higher than that of MAP-1 and MAP-2 (Muhlisin et al., 2014).

Yeast and Mold Count (YMC) The yeast and mold count of aerobic packaged chicken leg meat increased significantly ($P < 0.05$) during the storage period (Table 6). The YMC of the AP-CLM and MAP-CLM groups did not differ significantly ($P > 0.05$) during the refrigerated storage period. Also, there was no significant difference ($P > 0.05$) in the YMC values within MAP-CLM groups during the storage period.

Chicken samples contaminated by fungi are due to environmental contamination, since fungi are ubiquitous in water, air, soil, feeds, and processing materials (Greco et al., 2014). Freshly cut meat stored in a refrigerator with high humidity consistently undergoes microbial spoilage preferably mold spoilage. The **y**east and mold count varies between 1.87 and 2.52 log cfu/g in the fresh chicken meat sample (Santosh et al., 2012). Tuncer and Sireli (2008) reported yeast and mold count was 4.70 log cfu/g for the chicken meat packaged in a synthetic plate for 8 d and 6.07 log cfu/g for meat packaged in polythene for 10 d under refrigerated storage. The counts of yeasts and molds increased sharply from 0.2 log cfu/g to 4.44 log cfu/g on day 10 in normal air packaged roasted chicken leg samples, whereas the counts were inhibited significantly in MAPs (N: 100%N_2; M2: 20%CO_2/80%N_2; M3: 30%CO_2/70%N_2; M4: 40%CO_2/60%N_2) throughout the storage period, especially on CO_2−MAP treatment (Guo et al., 2018).

The initial yeast and mold count of wrap-packaged dry-aged beef were 2.60 and 2.86 log cfu/g. The number of yeast increased, but no change in mold growth was noticed during the 7 days storage period at 4°C (Lee et al., 2018). Significant effects ($P < 0.05$) were observed for MAP (MAP-1: 60%CO_2 + 40%N_2 and MAP-2: 80%CO_2 + 20%N_2) lamb meat under refrigeration for 25 d and the microbiological level of the yeast and mold in MAP-2 was lower than 5 cfu/g (Karabagias 2018).

As per Food Safety and Standards Regulations 2011, relating to microbiological requirements of food products, the Indian Standards IS:5403 states that the maximum permissible limit for yeast and mold count in chilled meat is 10^3 cfu/g. The chilled meat should be rejected as the yeast and mold count reaches/exceeds the level of 10^4 cfu/g (FSSAI, 2010). The results obtained in the current research regarding yeast and

Table 7. Sensory attribute changes in aerobic and modified atmosphere packaged chicken leg meat during refrigeration storage (4 ± 1° C).

Parameters/Groups	Days							
Appearance	0	3	6	9	12	15	18	21
AP-CLM	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.00 ± 0.01^{aB}	2.00 ± 0.01^{bC}	NA	NA	NA	NA
MAP-CLM20	5.00 ± 0.01^{aA}	4.34 ± 0.21^{bAB}	4.34 ± 0.21^{aAB}	4.67 ± 0.21^{aAB}	4.00 ± 0.37^{aB}	3.00 ± 0.37^{aC}	1.67 ± 0.21^{aD}	1.34 ± 0.21^{abD}
MAP-CLM10	5.00 ± 0.01^{aA}	4.34 ± 0.21^{bA}	4.67 ± 0.21^{aA}	4.67 ± 0.21^{aA}	3.34 ± 0.56^{aB}	3.00 ± 0.37^{aB}	1.34 ± 0.21^{abC}	1.67 ± 0.21^{aC}
MAP-CLM0	5.00 ± 0.01^{aA}	4.34 ± 0.21^{bA}	4.17 ± 0.40^{aAB}	4.34 ± 0.21^{aA}	3.34 ± 0.56^{aB}	3.34 ± 0.21^{aB}	1.34 ± 0.21^{abC}	1.34 ± 0.21^{abC}
Color								
AP-CLM	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.00 ± 0.01^{bB}	2.00 ± 0.01^{cC}	NA	NA	NA	NA
MAP-CLM20	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.67 ± 0.21^{aAB}	4.00 ± 0.01^{aBC}	3.97 ± 0.42^{aC}	3.67 ± 0.42^{aC}	1.67 ± 0.21^{aD}	1.34 ± 0.21^{aD}
MAP-CLM10	5.00 ± 0.01^{aA}	4.17 ± 0.11^{bB}	4.67 ± 0.21^{aAB}	4.00 ± 0.01^{aBC}	3.34 ± 0.56^{aCD}	3.00 ± 0.37^{aD}	1.34 ± 0.21^{abE}	1.33 ± 0.21^{aE}
MAP-CLM0	5.00 ± 0.01^{aA}	4.50 ± 0.32^{abAB}	3.84 ± 0.31^{bBC}	3.67 ± 0.21^{bBC}	3.34 ± 0.56^{aC}	3.34 ± 0.21^{aC}	1.34 ± 0.21^{abD}	1.34 ± 0.21^{aD}
Odor								
AP-CLM	5.00 ± 0.01^{aA}	4.00 ± 0.01^{bB}	3.84 ± 011^{bC}	2.00 ± 0.01^{bD}	NA	NA	NA	NA
MAP-CLM20	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.34 ± 0.21^{aB}	4.34 ± 0.21^{aB}	3.67 ± 0.21^{aC}	1.67 ± 0.42^{abD}	1.00 ± 0.01^{bE}
MAP-CLM10	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.34 ± 0.21^{aB}	3.67 ± 0.21^{bC}	3.67 ± 0.21^{aC}	1.67 ± 0.21^{abD}	2.00 ± 0.37^{aD}
MAP-CLM0	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.67 ± 0.21^{aA}	4.08 ± 0.08^{aB}	3.67 ± 0.21^{bB}	3.67 ± 0.21^{aB}	2.34 ± 0.21^{aC}	2.00 ± 0.37^{aC}
Sliminess								
AP-CLM	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.83 ± 0.11^{aB}	4.00 ± 0.01^{cC}	NA	NA	NA	NA
MAP-CLM20	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.67 ± 0.21^{aA}	2.67 ± 0.42^{aB}	2.00 ± 0.01^{aC}
MAP-CLM10	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.83 ± 0.11^{aA}	4.67 ± 0.21^{aA}	2.34 ± 0.56^{aB}	2.67 ± 0.42^{aB}
MAP-CLM0	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	5.00 ± 0.01^{aA}	4.83 ± 0.11^{bA}	4.67 ± 0.21^{aA}	4.67 ± 0.21^{aA}	2.67 ± 0.42^{aB}	2.67 ± 0.42^{aB}

n = 42; Means with different superscripts in the same column (small letters) and same row (capital letters) differ significantly ($P < 0.05$); AP-CLM = Aerobic packaged chicken leg meat; MAP-CLM20 = Modified atmosphere packaged chicken leg meat (20%O_2 + 30% CO_2+50%N_2); MAP-CLM10 = MAP leg meat (10%O_2 + 40% CO_2 + 50%N_2); MAP-CLM0 = MAP leg meat (0% O_2 + 20% CO_2 + 80% N_2) packaged at 4 ± 1°C; NA = Not Analyzed; 5-point descriptive scale (5 = Extremely desirable, 1 = Extremely undesirable)

mold count are comparable to the results of Santosh et al. (2012) and Guo et al. (2018). The chicken leg meat contamination was at a level similar to that seen in other studies by Santosh et al. (2012) and Guo et al. (2018).

Sensory Evaluation

The appearance, color, odor, and sliminess scores of the modified atmosphere-packed chicken leg meat decreased significantly ($P < 0.05$) during the storage period. The appearance score of the AP-CLM group was significantly ($P < 0.05$) lower than the MAP-CLM group on day 9 of the refrigerated storage period (Table 7). There was no significant difference ($P > 0.05$) in the appearance score within MAP-CLM groups during the whole storage period. The decrease in appearance may be due to the oxidation of myoglobin to metmyoglobin (Sahoo and Anjaneyulu, 1997).

The color score of the AP-CLM group was significantly ($P < 0.05$) lower than MAP-CLM groups on days 6 and 9 of the refrigerated storage period (Table 7). MAP-CLM10 had a significantly ($P < 0.05$) lower color score within MAP-CLM groups on day 3. The differences in the assessment of the color of chicken leg meat packaged in aerobic and modified atmosphere conditions showed that the decrease in the color of aerobic packaged chicken leg meat was positively correlated to the redness, total meat pigments, oxymyoglobin, metmyoglobin, deoxymyoglobin, and TBARS values during the storage period (Table 8). Whereas, the decrease in color scores of modified atmosphere packaged chicken leg meat were positively correlated to total meat pigments, and oxymyoglobin and negatively correlated to the redness, metmyoglobin, deoxymyoglobin, and TBARS values during the refrigerated storage (Table 9).

The odor score of the AP-CLM group was significantly ($P < 0.05$) lower than MAP-CLM groups on days 6 and 9 of the refrigerated storage period (Table 7). MAP-CLM20 had a significantly ($P < 0.05$) higher odor score on day 12 and a significantly ($P < 0.05$) lower odor score on day 21 within MAP-CLM groups.

The sliminess score of the AP-CLM group was significantly ($P < 0.05$) lower than the MAP-CLM group on day 9 of the refrigerated storage period (Table 7). MAP-CLM0 had a significantly ($P < 0.05$) lower slimness score on day 9.

The appearance was moderately acceptable up to day 9 in aerobic packages and all the MAP-CLM groups up

Table 8. Correlation between color parameters of aerobic packaged chicken leg meat during refrigeration storage (4 ± 1°C).

Color parameters	Sensory color	Redness	Total meat pigments	Oxymyoglobin	Metmyoglobin	Deoxymyoglobin	TBARS
Sensory color	1	0.941**	0.992**	0.948**	0.903**	0.797*	0.907**
Redness	0.941**	1	0.969**	0.993**	0.990**	0.932**	0.990***
Total meat pigments	0.992**	0.969**	1	0.979**	0.942**	0.849**	0.948**
Oxymyoglobin	0.948**	0.993**	0.979**	1	0.987**	0.929**	0.992**
Metmyoglobin	0.903**	0.990**	0.942**	0.987**	1	0.974**	0.998**
Deoxymyoglobin	0.907**	0.990**	0.948**	0.992**	0.998**	1	0.967**
TBARS	0.797*	0.932**	0.849**	0.929**	0.974**	0.967**	1

** $P < 0.01$.
* $P < 0.05$.

Table 9. Correlation between color parameters of modified atmosphere packaged (MAP = 10%O_2 + 40% CO_2 + 50%N_2) chicken leg meat during refrigeration storage (4 ± 1°C).

Color parameters	Sensory color	Redness	Total meat pigments	Oxymyoglobin	Metmyoglobin	Deoxymyoglobin	TBARS
Sensory color	1	−0.106	0.826*	0.888**	−0.932**	−0.926**	−0.789*
Redness	−0.106	1	0.39	−0.16	0.104	0.011	−0.306
Total meat pigments	0.826*	0.39	1	0.658	−0.811*	−0.744*	−0.932**
Oxymyoglobin	0.888**	−0.16	0.658	1	−0.914**	−0.969**	−0.652
Metmyoglobin	−0.932**	0.104	−0.811*	−0.914**	1	0.895**	0.827*
Deoxymyoglobin	−0.926**	0.011	−0.744*	−0.969**	0.895**	1	0.683
TBARS	−0.789*	−0.306	−0.932**	−0.652	0.827*	0.683	1

** $P < 0.01$.
* $P < 0.05$.

to day 15. The color was moderately acceptable up to day 6 in aerobic packages and MAP-CLM groups for up to 15 d. The slightly strange odor started from day 18 in MAP-CLM groups. The sliminess started from day 18 in MAP-CLM groups and in the aerobic packages, the sliminess was observed from day 9. Therefore, the current research indicated that the proposed composition of the gas mixture in MAP is O_2 up to 20%, CO_2 up to 40%, and N_2 up to 80% as possibly the best method of packaging from the point of view of the sensory acceptability of chicken leg meat at refrigerated storage.

The overall odor was described as more unpleasant and unacceptable in the argon MA from day 8 of storage as compared to day 12 in the oxygen MA. The general appearance of chicken skin was lighter in argon MA. The beginning of surface slime on the skin was recorded from day 12 of the storage in the oxygen MA, whereas in argon MA showed from day 16 of storage (Tomankova et al. 2012). Lee et al. (2018) noticed that the taste and odor of wrap-packaged dry-aged beef stored at 4°C for 7 days significantly deteriorated on day 5, while the overall acceptance significantly decreased on day 3 when compared to day 0.

CONCLUSION

The shelf-life of chicken leg meat analyzed in aerobic packaging under refrigerated conditions (4 ± 1°C) was 6 d. Based on chicken leg meat qualities such as reduction in TBARS value, higher oxymyoglobin, higher shear force denoting desirable firmness, delayed microbial proliferation, and delayed decline in sensory attributes such as appearance, color, odor, and sliminess, the modified atmosphere packaging of chicken leg meat indicated a shelf-life of 15 d at refrigerated storage irrespective of different gaseous concentrations. Hence, the modified atmosphere packaging allowed the shelf-life extension of the chicken leg meat by at least 9 d in comparison to aerobic packaging under refrigeration storage. For chicken leg meat, oxygen at the rate of 0 to 20% and carbon dioxide at the rate of 20 to 40% along with nitrogen gas at the rate of 50 to 80% are recommended in MAP for improving the shelf-life in refrigerated storage.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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