

Research Article

Shelf-life extension of roasted red chicken meat coloured with red mould rice by modified atmosphere packaging

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Abstract

The objective of this study was to determine the effect of modified atmosphere packaging (MAP) on quality and shelf-life of roasted red chicken meat coloured with red mould rice powder. Roasted red chicken meat was packed in air (control) and under MAP conditions (20%CO₂+ 80%N₂, M1; 40%CO₂+ 60%N₂, M2 and 60%CO₂+ 40%N₂, M3). All treatments were stored under refrigeration at 4±1°C. The results indicated that MAP conditions and storage time had no effects on water activity, moisture content, pH and surface colour of all samples (P>0.05) but had significant effects on texture and thiobarbituric acid values of roasted samples (P≤0.05). MAP was effective for inhibiting growth of total viable counts (TVC). The higher the CO₂ concentration, the higher the inhibition of microbial records. Yeasts and moulds, *Staphylococcus aureus*, *Escherichia coli* or coliforms were not detected in all samples throughout the storage period. Sensory analysis revealed that the products were better preserved under MAP conditions. Physicochemical, microbiological and sensorial data indicated that the shelf-life of aerobically packed roasted red chicken meat samples was 11 days. MAP could extend product shelf-life to 28 days under M1 and 49 days under M2 and M3.

Keywords: poultry, *Monascus purpureus*, red mould rice, shelf-life, MAP, storage

Introduction

Chicken meat products are popular worldwide due to their high nutritional quality and are available as either fresh or cooked products. However, chicken consumption has declined steadily due to the outbreak of avian influenza (A1) since early 2004. A variety of new products have had to be developed and introduced to the market, ranging from raw, ready-to-cook to semi-cooked or cooked chicken meat products [1, 2, 3, 4].

Modified atmosphere packaging (MAP) is one of the main technology developments in food packaging, which is designed to maintain the desired properties of food products for the desired period of storage and display. The technique is successfully used with various types of products, including fruit and vegetables, meat and meat products and seafood [5, 6, 7, 8]. The principle of MAP is the removal and/or replacement of packaged air with a different, fixed gas mixture. The proper combination of gases (CO₂, N₂ and O₂) in the headspace of food packs results in suppression of the microbial flora in stored food products, developed under aerobic conditions and retention of their sensorial attributes [9]. The success of MAP in extending food shelf life depends on many factors, including, good initial product quality, good hygiene during processing, correct packing material selection, appropriate gas mixture and maintenance of the process temperature [8]. A combination of different preservation techniques, such as chilling and gas packaging are used to achieve multi-target, mild but reliable preservation effects. Thus the objective of the present work was to study the effect of MAP including air on the shelf-life and quality of roasted red chicken meat coloured with *Monascus* red mould rice stored at 4±1⁰C using microbiological, physicochemical and sensory analyses.

Materials and Methods

Preparation of Monascus red rice powder

Jasmine rice, Pathumthani-1, was prepared at 25% moisture content and autoclaved at 121⁰C for 15 min. After cooling, 100 g of rice grains were inoculated with 5x10⁶ spores of *Monascus purpureus* TISTR 3002 and incubated at 30±2⁰C. After 14 days of incubation, rice grains were ground into fine powder using a mortar.

Preparation of roasted red chicken meat

Roasted red chicken meat was prepared according to a commercial recipe for roasted red pork. Skinless, boneless breasts of chicken were purchased from a local poultry processing plant. 500 g of chicken breast was mixed with curing ingredients (sucrose, 75 g ; table salt, 5.7 g; pepper, 1 tea spoon; garlic, 1 tea spoon; white soy source, 1 table spoon; black soy source, 1 table spoon). *Monascus* red rice powder was applied with various concentrations [1, 1.5, 2.0 and 2.5% (w/w)]. The curing meat samples were refrigerated (4±1⁰C) for an hour before roasting at 200±2⁰C for 20 min. Control samples were prepared in the same manner but applied with commercial roasted red pork colouring.

Preparation of roasted red chicken meat sample and MAP conditions

Roasted red chicken meat with the highest acceptance formulation was prepared as described above. After roasting and cooling, the samples (ca. 100g) were then placed on a polystyrene (PS) tray and packed in a nylon/LLDPE pouch (85 µm in thickness; oxygen transmission rate (OTR) of 101.4 cm³/m² day atm at 0% RH 23⁰C; carbon dioxide transmission rate (CO₂TR) of 74.5 cm³/m² day atm at 0% RH 23⁰C; and water vapor transmission rate of 1.64g/m² day at 100% RH 23⁰C. Gas mixtures were prepared by using the gas mixture (WITT MM-2G, Germany). The following MAP conditions were applied: 20%CO₂+80% N₂ (M1), 40%CO₂+60%N₂ (M2) and 60%CO₂+40%N₂ (M3). The gas concentrations in packages were monitored by gas analyzer (Servomex, Model 1450, UK). Pouches were heat-sealed using a vacuum sealer (Multivac C200, Germany) and kept at 4±1⁰C. For control, identical chicken samples were packed in air and kept under the same conditions. Samples from each treatment were randomly taken for microbiological, physicochemical and sensory analyses once a week throughout the storage period. The control samples were randomly taken at 2 day intervals.

Microbiological analysis

A sample (25g) was removed aseptically and transferred to a stomacher bag containing 225 ml sterile normal saline (0.85%NaCl) and homogenized using a stomacher (Smasher AES Laboratories, France) for 30s at room temperature. A 10-fold dilution was made of the normal saline as needed for plating. For microbial enumeration, 0.1 ml serial dilution samples were spread on the surface of dry media.

Total viable plate count (TVA) was enumerated on Plate Count Agar (PCA, Merck, Germany) and incubated at $30\pm 2^{\circ}\text{C}$ for 24 and 48 hr. Yeasts and moulds were enumerated using Potato Dextrose Agar (PDA, Merck, Germany) supplemented with chloramphenicol (100 $\mu\text{g/ml}$) after incubating at $30\pm 2^{\circ}\text{C}$ for 72 hr. *Escherichia coli* and coliform were determined on Petrifilm[™]. *E. coli* count and Petrifilm[™] rapid coliform count (3M, Germany), respectively after incubation at $35\pm 2^{\circ}\text{C}$ for 24hr. *Staphylococcus aureus* was determined using Baird-Parker medium (Biomark Laboratories, India) after incubation at $30\pm 2^{\circ}\text{C}$ for 48 hr. Enterobacteriaceae was determined by Violet Red Bile Glucose Agar (VRBGA, Merck, Germany) after incubation at $35\pm 2^{\circ}\text{C}$ for 48hr. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium. Suspected colonies were tested biochemically by the methods as described in the Food and Drug Administration Bacteriological Analytical Manual (<http://www.cfsan.gov/~ebam/bam-5.html>). Three replications of as least three appropriate dilutions were enumerated.

Physicochemical analysis

Water activity was determined using a_w meter (Novasina, TH-500, Switzerland). Samples from each replicate were ground into fine pieces before being measured. Moisture content was determined by oven drying of 5 g sample for 20-24 hr. according to AOAC [10]. The pH value was recorded using pH meter (Metrohm, Switzerland). Samples were thoroughly homogenized with 10 ml distilled water and the homogenate was used for pH determination. Thiobarbituric acid (TBA) was determined according to the method proposed by Pearson [11] and expressed as mg malonaldehyde/kg sample. Colour measurement was carried out on ten preselected locations of the sample surface. Sample was placed on the 1 inch diameter measuring area of a colour meter (Hunter Lab, Ultra Scan XE/IX7, USA) and results were expressed as L^* , a^* , b^* (CIELab units). Texture of the samples was determined using Texture analyser (TAX2i, USA). Two pieces from each sample were tested each time. Each piece was cut into 10 cubes of 1x1x2 cm. Each meat cube was cut perpendicular to the muscle fibres with a blade set with a knife probe (HDP/BSK). The testing conditions were; room temperature ($\sim 30^{\circ}\text{C}$); 2.0 mm/s test speed; 0.196N trigger point; 20 mm travel distance. Cutting force was measured and expressed as N/g.

Sensory evaluation

Sensory evaluation was performed in two sessions by a fifteen member trained panel who were trained for a period of 1 month to familiarize them with the sensorial attributes of roasted red chicken meat. In the first session, the appearance/colour of roasted red chicken meat coloured with different concentrations of *Monascus* red mould rice powder and the control sample were evaluated. Roasted red chicken meat samples were evaluated after being warmed up in a microwave oven at high power (700W) for 30 sec (surface temperature $\sim 40^{\circ}\text{C}$). Panelists evaluated the appearance/colour on a 5-point hedonic scale (1=extreme colouration to 5=excellent appearance). The acceptability score of 3.0 was taken as the lower limit of acceptability. The second session evaluated the storage samples. Panelists evaluated samples using a scale ranging from 1 to 9 in terms of colour (1= extreme discolouration due to microorganisms or chemical oxidation, etc. to 9 = natural roasted red chicken colour), odor (1 = extremely rancid to 9 = natural roasted red chicken odor), texture (1 = extremely mushy meat with soft crust to 9 = firm meat with crispy crust) and acceptability (1 = dislike very much to 9 =

like very much). A score of 5.0 was taken as the lower limit of acceptability. Prior to evaluation, samples were warmed to 40°C in a microwave oven. Along with the test samples, the panelists were presented with warmed roasted red chicken meat samples (~40°C), previously stored at -20°C throughout the experiment, this serving as the reference sample.

Statistical analysis

The collected data was analyzed based on ANOVA and presented as mean values with standard deviations. Duncan's multiple range test was used to determine significant differences between the mean values of treatments ($\alpha = 0.05$). All analyses were run in triplicate, except for colour and texture.

Results and Discussion

Roasted red chicken meat coloured with *Monascus red rice*

In this study *Monascus* red rice was used as a source of colour in roasted red chicken meat ingredients. Fermented red rice of *Monascus* fungus is the natural pigment that has been established as a food ingredient for Asian consumers and as a food colourant or spice in cooking such as rice wine, red soybean cheese, meat and fish products [12]. New food applications, such as the colouration of processed meat (sausage, hams), marine products (fish paste, surimi) and vegetable products (tomato sauce) have been described. Currently, more than 50 patents have been issued concerning the use of *Monascus* pigments for food [13].

In the preliminary study, two sets of roasted red chicken meat samples were compared. Commercial roasted red pork colouring was applied in one set and 1% (w/w) of *Monascus* red rice powder was used in another set. The result showed that the appearance/colour of roasted samples prepared with red mould rice powder was more or less close to conventional roasted chicken meat. In contrast, bright red colour was observed in samples prepared with commercial roasted red pork colouring, which was not accepted by the panelists. The following sets of roasted red chicken meat treated with red mould rice powder at different concentrations [1, 1.5, 2.0 and 2.5 (%w/w)] were prepared. Appearance/colour and the sensory qualities of the treatments are given in Figure 1 and Table 1, respectively. The results showed that the treatment coloured with 1.5 % (w/w) red mould rice powder gained the best colour, the best appearance and the highest acceptability.

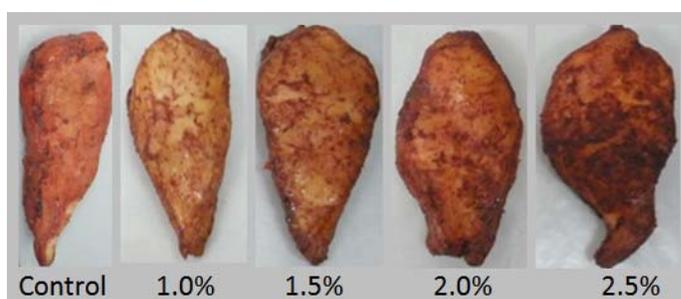


Figure 1. Appearance/colour of roasted red chicken breast coloured with commercial food colouring (control) and with *Monascus* red rice powder at different concentrations.

Table 1. Sensory qualities of roast red chicken meat coloured with different concentrations of red mould rice powder.

| Red mould rice powder (% w/w) | Colour* | Appearance* | Acceptability* |
|-------------------------------|---------------------------|---------------------------|--------------------------|
| 1.0 | 3.24 ^b ± 1.04 | 3.52 ^b ± 0.93 | 2.52 ^c ± 1.12 |
| 1.5 | 4.24 ^a ± 0.70 | 4.24 ^a ± 0.70 | 4.57 ^a ± 0.68 |
| 2.0 | 3.52 ^b ± 1.03 | 3.38 ^b ± 0.92 | 3.24 ^b ± 1.18 |
| 2.5 | 2.57 ^c ± 1.08 | 2.57 ^c ± 1.08 | 1.00 ^d ± 0.01 |
| Control | 3.05 ^{ab} ± 1.24 | 2.90 ^{bc} ± 1.22 | 3.01 ^b ± 1.51 |

* Means with different lower case letters (a, b...) in the same column are significantly different ($P \leq 0.05$)

MAP conditions and storage

Microbiological analysis

Roasted red chicken samples were monitored for TVC, yeasts and moulds, *Staphylococcus aureus*, *E. coli*, coliform and Enterobacteriaceae. At the initial stage, TVC of roasted samples was in the range of 5.01 ± 0.23 – 5.06 ± 0.08 log cfu/g and increased as the storage time proceeded (Fig. 2a). TVC of the samples packed in air and M1 increased progressively with storage time and reached the value of 6 log cfu/g, which is considered as the upper acceptability limit for cooked poultry meat as defined by ICMSF [14] on days 15 and 35 of storage, respectively. Therefore, TVC of air packed and M1 samples was not further determined after these days. The M2 and M3 samples did not reach this value throughout the 63 days of storage period under refrigeration. The results indicated that MAP consisting of 40% and 60% CO₂ was effective in extending the lag phase or reducing the growth rate of microorganisms. Since carbon dioxide (CO₂) has bacteriostatic and fungistatic properties, the higher the CO₂ concentration the lower the microorganism's respiration and growth rate [8, 15]. This observation was in agreement with the previous report by Patsias, *et al* [3], who found that CO₂ showed a spoilage delay of precooked fried chicken by inhibiting the growth of microorganisms.

Yeasts and moulds although strictly aerobic, were not detected in all treatments including air-packed samples. Gram positive bacteria, *Staphylococcus aureus*, are facultative or strict anaerobes [16] that are generally more tolerant to CO₂ than Gram negative bacteria *E. coli*, coliform or Enterobacteriaceae [17]. However, none of these microorganisms were detected in all samples throughout the storage period. This might be because these microorganisms are sensitive to extrinsic factors such as heat [18]. Therefore, roasting the chicken samples at 200^oC for 20 min. is sufficient to eliminate these microorganisms.

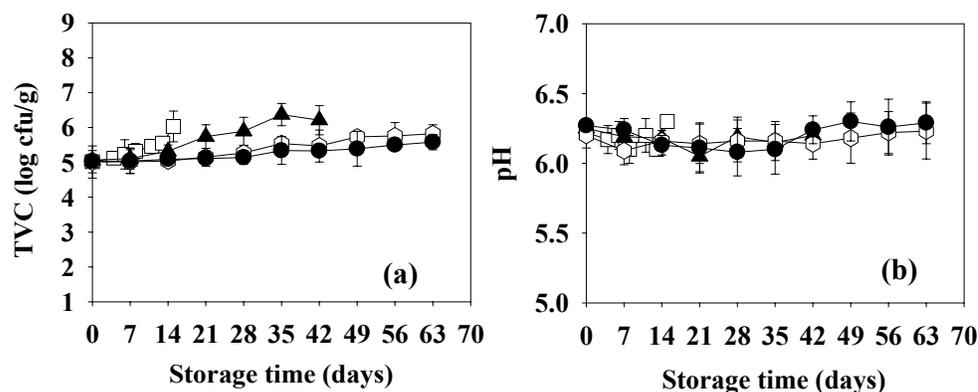


Figure 2. Changes in total viable count (log cfu/g) (a) and pH (b) of roasted red chicken meat packed in air and under MAP conditions (M1 = 20%CO₂+80%N₂, M2 = 40%CO₂+60% N₂ and M3 = 60%CO₂+40%N₂). (Air, □; M1,▲; M2○; M3●)

Physicochemical analysis

Gas mixture conditions and storage time did not have significant effects ($P>0.05$) on pH of the stored samples (Fig. 2b). The pH value of the samples was 6.24 ± 0.03 at the beginning and was 6.31-6.40 after 63 days of storage. This pH range enhanced the growth of contaminating microorganisms on storage samples (Fig. 2a). Normally, the higher the CO_2 concentration, the higher dissolution of CO_2 reducing the pH of food. A slightly increase of pH in this study could be due to a balance between the dissolution of CO_2 and the deamination reaction of amino acids, which derived from the hydrolysis of protein during the growth of microorganisms [15]. In addition, the solubility of CO_2 in water decreases with the increase of NaCl concentration [19]. Since sugar, NaCl and soy source were applied to the product as ingredients, therefore the solubility of CO_2 in MAP sample was reduced.

Figure 3 shows the changes in water activity and moisture content during storage of roasted chicken meat samples both in air and under MAP conditions at $4\pm 1^\circ\text{C}$. At the beginning, water activity of all samples was as low as 0.93 ± 0.02 (Figure 3a), due to the compositions of roasted meat ingredients used in this study. Water activity and moisture content of all samples slightly increased but were not significantly different from the initial ($P>0.05$). The values were in the ranges of 0.93-0.96 and 61.29-62.68%, respectively. The gradual increase of moisture and water activity of all samples could be due to moisture absorption by the food from the environment that gradually permeated through packaging materials and also from the respiration of the growing microorganisms [20].

TBA values of roasted red chicken meat are presented in Figure 4a. At the beginning, TBA of all roasted samples was at 1.28 ± 0.14 mg malonaldehyde/kg sample. The TBA values of all samples increased as storage time proceeded. TBA values of roasted red chicken meat in air packages increased faster than those under MAP packaged conditions, M1, M2 and M3, respectively. At 15 days of storage, the TBA value of samples in air-packages was at 6.85 ± 0.85 mg malonaldehyde/kg sample, whereas those packed under MAP conditions showed TBA values within the range of 2.90-3.80 mg malonaldehyde/kg sample during the same period. This observation indicates that the presence of oxygen in the package is a critical factor influencing lipid oxidation [21]. The increment of CO_2 from 20% (M1) to 60% (M3) significantly affected this parameter ($P\leq 0.05$). After 15 days of storing, TBA of air packed samples was not determined since the TVC value reached the limiting threshold (10^6cfu/g) for cooked poultry meat (Fig. 2a). Also the samples packed under M1 condition, the end of sampling time was 42 days, when TVA reached the limiting threshold. TBA value of M1 sample at 42 days storage was 13.59 ± 0.71 mg malonaldehyde/kg sample. At the end of the experiment at 63 days of storage, TBA values of samples packed under M2 and M3 conditions were 11.80 ± 0.11 and 11.43 ± 0.53 mg malonaldehyde/kg sample, respectively. The gradual increase of TBA values of the samples packed under MAP conditions resulted from O_2 , which permeated through packaging materials (data not shown).

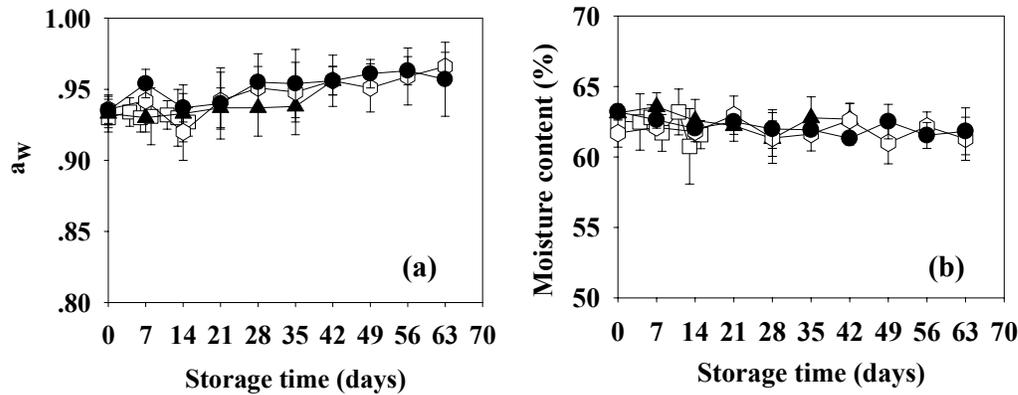


Figure 3. Changes in water activity (a) and moisture content (b) of chilled roasted red chicken meat packed in air and under MAP conditions (M1 = 20%CO₂ + 80%N₂, M2 = 40%CO₂ + 60%N₂ and M3 = 60%CO₂ + 40%N₂). (Air, □; M1,▲; M2,○; M3,●)

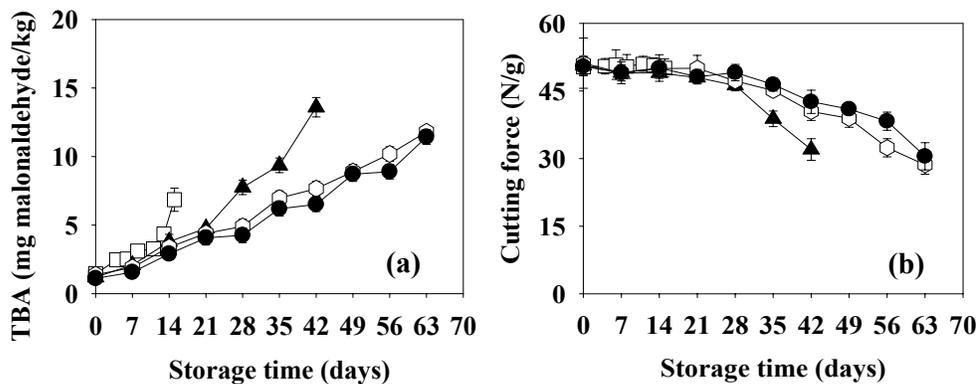
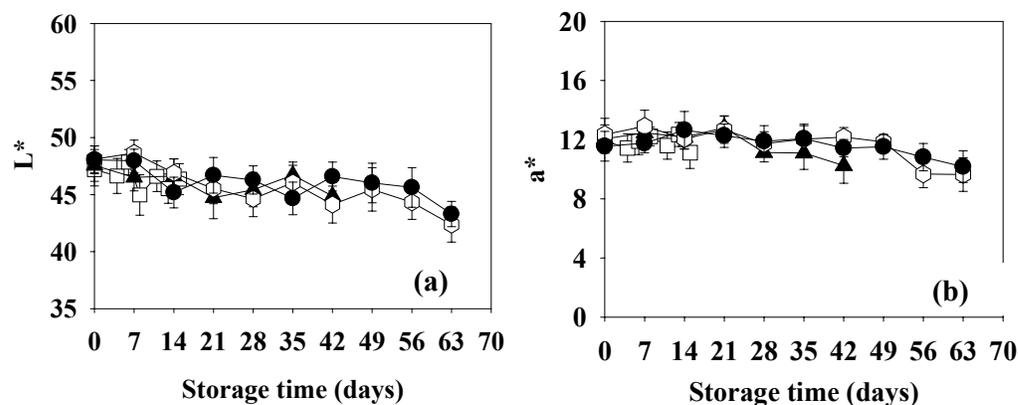


Figure 4. Change in thiobarbituric acid (TBA) value (a) and texture, cutting force (b) of chilled roasted red chicken meat packed in air and under MAP conditions (M1 = 20%CO₂ + 80%N₂, M2 = 40%CO₂ + 60%N₂ and M3 = 60%CO₂ + 40%N₂). (Air, □; M1,▲; M2,○; M3,●)

Texture of roasted samples, which was represented as cutting force are presented in Figure 4b. Cutting force was 50.63 ± 0.33 N/g at the initial and did not obviously change during 15 and 28 days of air packaged and MAP conditions, respectively. After that, less force is needed to shear MAP samples. These results indicated that both gas mixture conditions and storage time had significant effects on cutting force ($P \leq 0.05$).



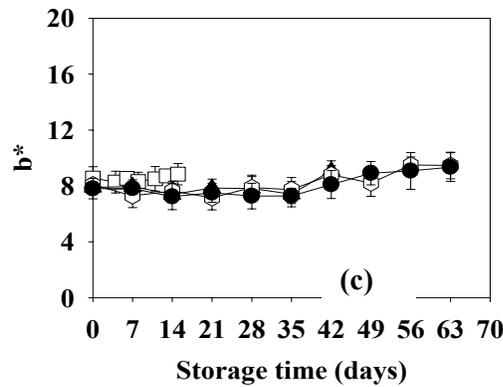
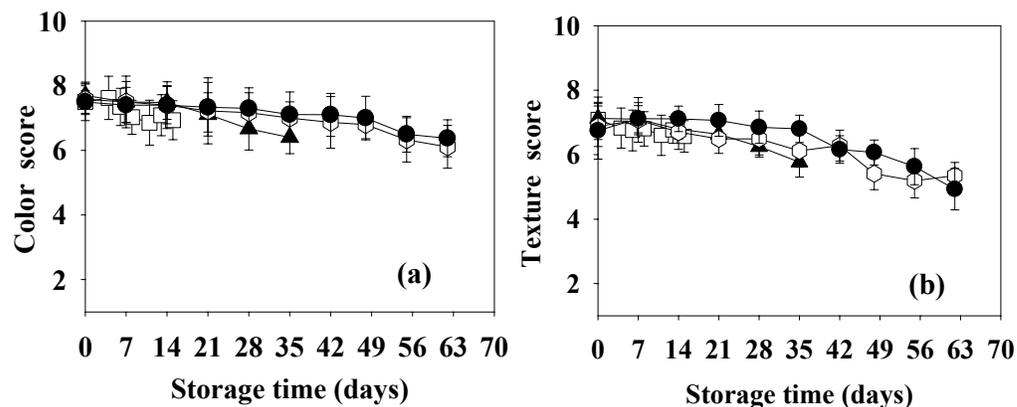


Figure 5. Change in colour, L*(a), a*(b) and b*(c) of chilled roasted red chicken meat packed in air and under MAP conditions (M1 = 20%CO₂ + 80%N₂, M2 = 40%CO₂ + 60%N₂ and M3 = 60%CO₂ + 40%N₂). (Air, □; M1,▲; M2,◇; M3,●)

The colour values as L*, a* and b* are shown in Figure 5. L*, a* and b* values of air packed and M1 samples were stable during 15 and 42 days storage, respectively. At the late stage of storage, L* and a* values of samples packed under M2 & M3 conditions decreased. In contrast, b* value increased but did not significantly change (P>0.05). The results indicated that colour of the product became pale and dull as the storage time progressed. However, MAP had no significant effect on the colour of roasted red chicken meat samples (P>0.05).

Sensory evaluation

Sensory scores of roasted samples in terms of colour, texture and odour decreased as storage time proceeded (Fig. 6). A significant decrease in acceptability score of samples packed in air and under M1 conditions was mainly due to rancid odour or odourless. Odour of roasted red chicken meat samples is a complex attribute originating from lipid and peptide components of roasted meat and the components of the curing ingredients, which was the most important parameter affecting panelists acceptability on roasted samples. On the other hand, the decrement of acceptability scores of samples packed under M2 and M3, which gradually decreased during storage time was mainly due to looser texture and rancid odour. The results agreed well with the changes in TVC (Fig. 2a) and TBA (Fig. 4a) values. MAP had no significant effect on the colour of roasted red chicken meat samples (P>0.05). This result indicated that the colour of roasted red chicken meat coloured with *Monascus* red rice was quite stable under refrigeration.



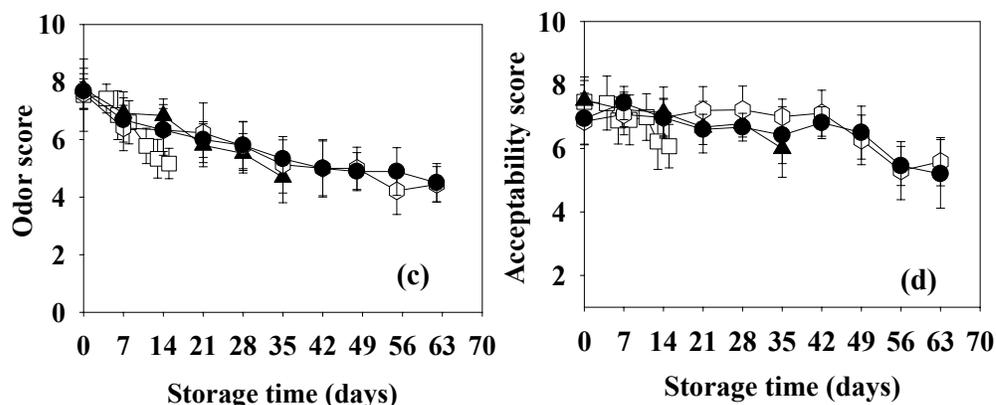


Figure 6. Colour score (a); texture score (b); odour score (c); and acceptability score (d) of chilled roasted red chicken meat packed in air and under MAP conditions (M1 = 20%CO₂ + 80%N₂, M2 = 40%CO₂ + 60%N₂ and M3 = 60%CO₂ + 40%N₂). (Air, □; M1, ▲; M2, ○; M3, ●)

Conclusions

The limiting factors for roasted red chicken meat samples packed in air and M1 package was microbial spoilage, whereas for those in M2 and M3 it was lipid oxidation and looser texture. Based on microbiological, physicochemical, and sensorial properties, the shelf-life of roasted red chicken meat coloured with *Monascus* red mould rice powder was 11 days. Modified atmosphere packaging could extend product shelf-life to 28 days under M1 and 49 days under M2 and M3 conditions.

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