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Microbiological and Physicochemical Changes in Rabbit Meat During Ambient Storage

Marielle D. Gamboa[1,2,*], Dennis Marvin O. Santiago[2], April Shayne L. Sulabo[2], and Mary Ann O. Torio[3]

[1] Institute of Engineering and Applied Technology, Bulacan Agricultural State College, San Ildefonso, Bulacan, 3010 Philippines
[2] Institute of Food Science and Technology, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, 4031 Philippines
[3] Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños, College, Laguna, 4031 Philippines

*Author for correspondence: Email: mdgamboa01@gmail.com; marielle_gamboa@basc.edu.ph

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This study aimed to evaluate the changes in rabbit meat during storage at ambient temperature (30 ± 1ºC) for nine hours. The study focused on two rabbit muscle parts: the hind leg and the saddle, investigated in terms of microbiological parameters such as aerobic plate count (APC) and total coliform count (TCC); and physicochemical parameters such as pH, color, total myoglobin concentration, and lipid oxidation. Data obtained were subjected to statistical analysis at a 5% significance level ($p < 0.05$). Results revealed that the pH of both portions of rabbit muscle decreases over time during storage. This decrease is attributed to the growth of acid-producing microorganisms, which ultimately leads to spoilage in rabbit meat. On the contrary, color values (L*, a*, and b*) showed an increasing trend in both muscle portions, indicating a discoloration of rabbit meat during storage. Both lipid oxidation and total myoglobin content of rabbit meat did not change significantly during storage, but lipid oxidation varied between muscle parts. Based on the microbiological analysis of rabbit meat, both aerobic plate count (APC) and total coliform count (TCC) increased significantly during storage in both muscle portions. Results of the study suggest that freshly slaughtered rabbit meat must remain at ambient storage for the shortest time, as this storage condition will cause spoilage of meat, making it unfit for human consumption.

Keywords: rabbit meat, pH, shelf-life, ambient, color

INTRODUCTION

Rabbit meat has been long accepted as a meat source by Western countries and some Asian countries, particularly China and Korea. The market for rabbit meat in these countries is relatively high. In fact, rabbit meat ranked 4th behind beef, pork, and poultry (FAO 2019). In the Philippines, the consumption of rabbit meat was introduced during the COVID-19 pandemic and the African Swine Fever (ASF) outbreak, where people do not have access to enough meat. This shortage in meat drives market prices to increase, thus preventing Filipinos from consuming high-quality, protein-rich food. One of the government initiatives to address this issue was introducing rabbit farming. Rabbits, with their fast growth rate, can be slaughtered and become a source of meat within 10 – 12 wk. Furthermore, rabbit meat has a higher nutritional value than other varieties of meat such as pork, chicken, and beef, owing to its lower levels of crude fat, sodium, and cholesterol (Nistor et al. 2013). Rabbit meat also has higher proportions of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids, both recognized for their health-promoting benefits. These benefits include reduced cholesterol and inflammation as well as and decreased risk of chronic diseases such as heart disease, cancer, and arthritis (López-Miranda et al. 2006; Wall et al., 2010; Gammone et al. 2018).

Through the continuous support of the Philippine government, rabbit meat will soon be available in local wet markets. Consequently, it is necessary to determine the changes that may occur during its post-mortem storage. In the

Philippines, it is customary for freshly butchered meat to be handled, stored at room temperature in wet markets, and sold within the day during the stipulated period by the National Meat Inspection Service (DA-NMIS 2012). This protocol is attributed to the high water and nutrient content of meat and its near-neutral pH, rendering it susceptible to microbial attacks and enzymatic reactions. Improper handling of meat can result in both food spoilage and food-borne illnesses.

Fresh meat stored at ambient temperature has a limited shelf life; hence, it must be handled and transported at low temperatures (Koutsoumanis et al. 2006). Handling meat at low temperatures would prevent the proliferation of microorganisms, which is the primary determinant of meat quality. High microbial load in meat produces off-odors and -flavors and can alter the meat's physicochemical composition, including but not limited to its color, pH, and water-holding capacity. According to BAFS (2023), meat is considered spoiled and unsaleable if the total aerobic count reaches 5 x 10^6 CFU g^{-1}. Likewise, the development of off-odors associated with oxidative rancidity in meat like chicken and pork can be detected when the concentration of malondialdehyde (MDA) in mg per kg meat becomes 0.9 and 1.0, respectively (McKenna et al. 2005; EOS 2006).

Since meat freshness is the primary criterion that drives customer purchase decisions, it is critical to establish the end of its shelf-life and characterize the changes during storage. Assessing the quality changes in rabbit meat at ambient temperature would be very beneficial since this is a widespread practice among the general public. Thus, this study evaluated the microbiological and physicochemical changes in rabbit meat during ambient storage conditions.

MATERIALS AND METHODS

Slaughtering of Rabbits

A total of 6 male upgraded New Zealand White (NZW) rabbits with an average age of 14 ± 1 wk and live body weight of 2.3 ± 0.2 kg were used in this study. The rabbits were fed *ad libitum* with forage and water and commercial pellet feeds twice a day. The rabbits were slaughtered following the Code of Practice for slaughtering rabbits (EFSA AHAW Panel et al 2020; BAFS 2022) after a 12-h fasting period under hygienic conditions. The hot carcasses were suspended in a ventilated area for 30 min, placed in an iced box, and transported to the Institute of Food Science and Technology, College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB) within 45 min. The rabbit carcasses were vacuum-packed and stored for 4 h at 4ºC before fabrication. Two portions of the rabbit carcass—the hind leg and the saddle—were considered since these comprised the major portion of the carcass. The saddle refers to the meat that runs along the back of the rabbit, including the loin and the muscles on either side of the spine. This part of the rabbit meat covers two muscles, namely the *longissimus dorsi* and *iliocostalis*. The microbiological and physicochemical changes in both muscle portions were then observed every hour of storage at ambient temperature, 30 ± 1ºC.

Preparation of Meat Samples

Rabbit carcasses were portioned into retail cuts under hygienic conditions. Hind leg and saddle muscle samples were individually placed in foam trays with absorbent pads and overwrapped with polyvinyl chloride (PVC) plastic film with a thickness of 9 – 20 microns. The samples were stored in a room with an ambient temperature of 30 ± 1ºC.

For the determination of lipid oxidation and total myoglobin content, a 15-g sample was collected every hour until 9 h of storage for each rabbit portion. The sample was collected 1 cm from the surface of the meat, wrapped in aluminum foil, and rapidly frozen in liquid nitrogen. The meat samples were stored in a polyethylene sealable plastic at 0ºC and analyzed within 30 d. Frozen meat samples were ground before determining lipid oxidation and total myoglobin content.

Physicochemical Analyses

Color

The color of the meat was measured using a handheld chromameter (CR-400 Konica Minolta). The parameters measured were lightness (L*), redness (a*), and yellowness (b*). The measurements were taken under diffuse illumination and an 8-mm diameter aperture, using a D65 light source. Before taking the measurements, the instrument was calibrated with a white standard plate. A total of three readings were taken from each meat sample every hour for up to 9 h of storage. The average color measurements were recorded for each sample. The hue angle and chroma were also calculated according to the guidelines of the American Meat Science Association (AMSA 2012).

pH

The pH of the meat was determined by using a calibrated spear-type pH electrode (YY-1030 Yierye) which was inserted into the meat sample. An average of three pH readings was taken at different locations for each meat sample. The pH was measured hourly for up to 9 h of storage.

Total Myoglobin Content

The following method was used to determine the total myoglobin content of rabbit meat samples with slight modifications to the AMSA (2012) method. One gram of

ground meat sample was mixed with 5 mL ice-cold 40-mM potassium phosphate buffer, pH 6.8 in 15-mL polypropylene tubes. The sample was mixed thoroughly and placed at 0 – 4ºC for 1-h extraction of myoglobin. After extraction, the sample was centrifuged at 5000 rpm for 30 min at 4ºC (Lee et al. 1999). The supernatant was filtered using Whatman No. 1. The absorbance of the supernatant was measured at 525 nm, which is the isosbestic point for the three forms of myoglobin, using UV-Vis Spectrophotometer (UV-1900 Shimadzu) against a blank. The total myoglobin concentration was calculated following the formula of AMSA (2012) and expressed as milligrams of myoglobin per gram of meat.

Lipid Oxidation

The Thiobarbituric Acid Reactive Substances (TBARS) assay is a method used to determine the extent of lipid oxidation in meat, which serves as an indicator of its deterioration. A sample of 0.5 g of meat was mixed with a 2.5-mL solution consisting of 0.375% thiobarbituric acid (TBA), 15% trichloroacetic acid (TCA), and 0.25 N hydrochloric acid (HCl). The mixture was incubated in a water bath at 95ºC for 15 min, cooled with running water, and centrifuged at 5500 rpm, at 4ºC for 25 min. The supernatant was collected, and absorbance at 532 nm was measured using a UV-Vis Spectrophotometer (UV-1900 Shimadzu) against a blank. The TBARS value was computed based on AMSA (2012) and expressed as mg malonaldehyde/kg wet sample.

Microbiological Analyses

The aerobic plate count (APC) and total coliform count (TCC) of meat samples were determined every hour up to 9 h of storage at 30 ± 1ºC. A 10-g sample was mixed with 90 mL of 0.1% sterile peptone water, and the mixture was serially diluted up to 10^7. One mL of each dilution was plated in duplicate. For APC determination, 15 mL of Plate Count Agar (PCA) was poured onto the plates and for total coliform determination, Violet Red Bile Agar (VRBA) was used. The inoculated plates were incubated for 18 – 24 h for coliform and 48 h for APC at 35ºC. Colonies that grew on VRBA were confirmed by picking 10 representative colonies and transferring each to a tube containing Brilliant Green Lactose Bile (BGLB) broth, which was then incubated at 35ºC for 24 – 48 h for gas production (Maturin and Peeler 2001). The microbial count was calculated based on the formula of Maturin and Peeler (2001), considering only those plates with valid counts (25 – 250 colonies).

Experimental Design and Statistical Analysis

The experiment followed a Completely Randomized Design (CRD) with storage hours as fixed effects. Three treatment replicates were done for all the analyses. The samples (hind leg and saddle) used in each replicate were taken from the two rabbit carcasses and then stored at ambient conditions. During the analysis, a portion of each muscle was taken as a sample every hour, for up to 9 h storage. Analyses include pH, color, total myoglobin content, lipid oxidation, aerobic plate count, and total coliform count. In each analysis, three readings were obtained except for microbiological analyses with duplicate readings. Microbial counts were transformed into logarithmic scales before analysis. Means following Gaussian distribution were analyzed using PROC GLM of Minitab version 17.1.0 with Tukey's honest significant difference as post hoc test at $p < 0.05$. Means following non-Gaussian distribution were analyzed using Kruskal-Wallis test of XLSTAT version 2021.2 with Dunn's test as post hoc test. Kendall's Tau correlation coefficient was used to describe the relationship between measured parameters at $p < 0.05$.

RESULTS AND DISCUSSION

pH

Table 1 shows the pH changes in rabbit meat during storage. The pH of rabbit meat decreased with storage at ambient conditions. The initial pH of the hind leg and saddle were 6.08 ± 0.01 and 5.97 ± 0.01, respectively. The pH values in both rabbit muscle portions are comparable to the acceptable pH in freshly slaughtered meat. Newly slaughtered meat typically has near-neutral pH. This is mainly because the pH of living muscle is within pH 7.0 – 7.2 for supporting normal metabolism and maintaining homeostasis. When an animal is slaughtered, the oxygen supply ceases, thus stopping the oxidation processes for its normal metabolism. The muscle then shifts to an anaerobic process to maintain its homeostatic condition. As a result, energy in the form of adenosine

Table 1. Effect of ambient temperature on pH of rabbit meat during storage.

Storage Time (h)	pH	
	Hind Leg	Saddle
Initial	6.08 ± 0.01^{aA}	5.97 ± 0.01^{aB}
1	6.02 ± 0.03^{aA}	5.86 ± 0.07^{abB}
2	5.84 ± 0.01^{b}	5.81 ± 0.07^{ab}
3	5.80 ± 0.01^{bc}	5.73 ± 0.07^{ab}
4	5.75 ± 0.04^{bc}	5.70 ± 0.06^{ab}
5	5.71 ± 0.02^{c}	5.65 ± 0.06^{ab}
6	5.69 ± 0.02^{cA}	5.63 ± 0.02^{bB}
7	5.67 ± 0.02^{cA}	5.60 ± 0.02^{bB}
8	5.64 ± 0.00^{cA}	5.59 ± 0.00^{bB}
9	5.64 ± 0.01^{cA}	5.54 ± 0.01^{bB}

Values are presented as mean ± standard deviation (n = 3);
A, B Values with different letter superscripts within row marks significant difference between muscle portions at $p < 0.05$;
a, b…Values with different letter superscripts within the column marks significant difference between the storage time at $p < 0.05$.

triphosphate (ATP) is synthesized from the stored glycogen until all supplies are depleted. These processes will produce hydrogen ions and lactate from ATP hydrolysis and glycolysis, respectively. The hydrogen ions will then react with lactate to form lactic acid. Thus, continuous glycogen degradation can lead to the accumulation of lactic acid in the muscle, which then causes its pH to decrease over time (Toldra 2017).

The decrease in pH due to muscle acidification can last up to 24 h; thus, the pH of the meat after 24 h is usually considered the ultimate pH, which is often the basis for meat quality. The ultimate pH (pH_u) varies across meat types. Fresh pork, chicken, and beef have a pH_u of 5.42 – 6.26 (Holmer et al. 2009), 5.7 – 6.1 (Beauclercq et al. 2022), and 5.8 – 6.2 (Hamoen et al. 2013), respectively. In rabbit meat, the ultimate is pH_u 5.72 – 5.90 (De Liu et al. 2012; Kozioł et al. 2016; Maj et al. 2016; Wang et al. 2016; Perna et al. 2019; Menchetti et al. 2020).

A consistent decrease in pH was noted in both muscle portions of rabbit meat. This decline is likely linked to the proliferation of acid-producing microorganisms, including lactic acid bacteria such as *Leuconostoc*/*Weissella* sp., *Enterococcu*s, and coliforms (Kalschne et al. 2015). Similar results were obtained in the study of Manalo and Gabriel (2020) in pork and chicken stored at ambient temperature. The pH of pork and chicken meat declined from 6.22 and 6.15 to 5.97 and 5.85, respectively, attributed to the increasing organic acid shown in the % titratable acidity of the meat samples. In addition, the initial microbial load and its metabolism can also affect the rate of pH reduction in meat.

The pH of the different rabbit muscle portions—the hind leg and the saddle—also differed significantly, indicating that different muscles have varying rates of pH decline during post-mortem storage. The varying decline in muscle pH can be ascribed to the predominant composition of fibers in each muscle. The types of fibers in the muscle are categorized based on their contractile and metabolic characteristics. Generally, the skeletal muscle fiber consists of four different types: Type I (slow-oxidative), Type II (fast oxido-glycolytic) and Type IIX and IIB (fast glycolytic) (Schiaffino and Reggiani 2011). This is a modification of the earlier classification by Ashmore and Doerr (1971): αR (red, rapidly contractile), βR (red, slow contractile), and αW (white, rapidly contractile). A higher proportion of Type II fibers (W) signifies rapid glycolysis, resulting in an accelerated pH decline in the muscle during post-mortem storage (Joo et al. 2013). *Longissimus lumborum* has a higher percentage of Type II fibers (αW) consisting of 90.7 – 94.9% in different breeds of rabbit meat (Tůmová et al. 2014), thus explaining the faster decline in pH of the saddle muscle during storage. Dalle Zotte et al. (2016) also found higher proportions of αW (81%) than αR (12.9%) and βR (6.2%) in the same muscle of rabbit.

Color

The color of rabbit meat is measured in terms of L* (blackness–whiteness), a* (greenness–redness), and b* (blueness–yellowness) values. Color in meat is known to be an index of freshness and safety, which drives the consumer's purchase intention. Hence, information on the color changes of rabbit meat during storage is vital as it suggests the timeframe at which meat will become unacceptable to consumers.

Table 2 depicts the color values (L*, a*, b*) of rabbit meat during storage. All color parameters significantly increased with storage time in both rabbit muscle portions, and values obtained significantly differed between portions. Previous research suggests that the muscles that perform harder work have higher myoglobin content than the muscles of the back (Kozioł et al. 2015). This was reflected in the results of this study, where the hind leg of rabbit meat had significantly lower L* values than the saddle portions but had higher a* and b* values.

Meat discoloration can indicate spoilage due to prolonged storage. However, the correlation between L*, a *, and b* values and discoloration in meat varies across research. For instance, in the study of Olivera et al. (2013), discoloration of beef samples is indicated by decreased a* values at varying temperatures and conditions. The decline was more evident in meat exposed at higher temperatures aerobically. On the other hand, prolonged storage of chicken breast resulted in an upward trend in L*, a*, and b* values. It was shown that meat stored at high temperatures (36°C) caused an increase in all color parameters. However, elevated storage temperature decreased color values (Zhang et al. 2019). The latter study supported the results of this study, where color values significantly increased over time, indicating discoloration of the meat. Hence, this denotes that temperature is a factor that affects the color of fresh meat.

Aside from oxygen, which is known to be responsible for oxidizing the ferrous iron in the heme compound to ferric, forming the brown pigment (metmyoglobin), another major factor that causes a significant change in the color of fresh meat is the growth of microorganisms. Aerobic microorganisms that thrive in meat include yeasts, *Pseudomonas* spp., *Acinetobacter liquefaciens*, and *Flavobacterium rhenanus*, which utilize oxygen to metabolize nutrients. Products of their metabolism include gases, which then affect the partial pressure in the meat. When the oxygen is reduced to critical pressures, the concentration of metmyoglobin will increase, leading to a color change in meat from red to brown to purple. Moreover, microorganisms such as *Pseudomonas* and *Leuconostoc* (e.g., *Leuconostoc citreum* and *Leuconostoc gelidum*) are known to produce green pigments from hydrogen sulfide (Robach and Costilow 1961) and yellow pigments, respectively, which alters meat color (Kröckel 2013; Toldra 2017).

Table 2. Effect of ambient temperature on color values (L*, a* and b*) of rabbit meat during storage.

Storage Time (h)	L* (Lightness)		a* (Redness)		b* (Yellowness)	
	Hind Leg	Saddle	Hind Leg	Saddle	Hind Leg	Saddle
Initial	52.53 ± 0.68^{eB}	55.06 ± 0.41^{fA}	1.64 ± 0.29^{cA}	0.65 ± 0.02^{gB}	1.18 ± 0.09^{bA}	-2.87 ± 0.11^{aB}
1	53.32 ± 0.66^{deB}	55.86 ± 0.59^{efA}	1.77 ± 0.28^{cA}	0.84 ± 0.10^{fgB}	1.42 ± 0.13^{bA}	-1.90 ± 0.10^{abB}
2	53.82 ± 0.47^{cdeB}	56.48 ± 0.79^{efA}	1.94 ± 0.29^{cA}	0.92 ± 0.21^{efgB}	1.66 ± 0.23^{bA}	-1.64 ± 0.06^{abcB}
3	54.20 ± 0.49^{bcdeB}	57.43 ± 0.87^{defA}	2.07 ± 0.35^{cA}	1.34 ± 0.15^{defB}	1.99 ± 0.07^{b}	-1.42 ± 0.10^{abc}
4	54.60 ± 0.58^{bcdeB}	58.16 ± 0.94^{cdefA}	2.13 ± 0.38^{cA}	1.46 ± 0.16^{deB}	2.29 ± 0.27^{ab}	-1.34 ± 0.16^{abc}
5	55.68 ± 1.03^{bcdB}	59.07 ± 1.38^{bcdeA}	2.34 ± 0.19^{bcA}	1.72 ± 0.12^{cdB}	2.39 ± 0.26^{ab}	-1.09 ± 0.81^{abc}
6	55.98 ± 1.07^{abcdB}	60.52 ± 0.84^{bcdA}	3.09 ± 0.19^{abA}	2.28 ± 0.19^{bcB}	2.67 ± 0.34^{abA}	-0.66 ± 0.55^{abcB}
7	56.36 ± 1.06^{abcB}	61.54 ± 0.75^{abcA}	3.48 ± 0.20^{aA}	2.37 ± 0.20^{bB}	3.01 ± 0.40^{abA}	0.63 ± 0.86^{abcB}
8	57.03 ± 1.11^{abB}	62.20 ± 0.83^{abA}	3.76 ± 0.07^{aA}	2.69 ± 0.27^{abB}	3.35 ± 0.11^{aA}	1.42 ± 0.02^{bcB}
9	58.81 ± 0.51^{aB}	64.38 ± 2.06^{aA}	3.85 ± 0.11^{aA}	3.06 ± 0.03^{aB}	3.59 ± 0.22^{aA}	1.67 ± 0.11^{cB}

Values are presented as mean ± standard deviation (n = 3);
A, B Values with different letter superscripts within row marks significant difference between muscle portions at $p < 0.05$;
a, b…Values with different letter superscripts within the column marks significant difference between the storage time at $p < 0.05$.

Table 3. Effect of ambient temperature on the chroma and hue angle of rabbit meat during storage.

Storage Time (h)	Chroma		Hue Angle	
	Hind Leg	Saddle	Hind Leg	Saddle
Initial	2.03 ± 0.28^{eB}	2.90 ± 0.12^{bcA}	36.34 ± 6.6^{B}	283.10 ± 0.30^{aA}
1	2.28 ± 0.20^{eA}	2.04 ± 0.10^{cdeB}	39.30 ± 7.9^{B}	296.30 ± 3.2^{abA}
2	2.57 ± 0.24^{eA}	1.92 ± 0.16^{eB}	40.77 ± 8.9^{B}	301.63 ± 5.6^{abA}
3	2.88 ± 0.36^{deA}	2.00 ± 0.13^{deB}	44.40 ± 5.3^{B}	310.57 ± 4.6^{abA}
4	3.13 ± 0.56^{cdeA}	2.01 ± 0.18^{deB}	47.34 ± 2.8^{B}	317.53 ± 5.1^{abA}
5	3.35 ± 0.39^{cdeA}	1.96 ± 0.16^{eB}	45.54 ± 1.8^{B}	329.17 ± 2.5^{abA}
6	4.11 ± 0.09^{cdA}	2.38 ± 0.27^{cdeB}	40.75 ± 6.6^{B}	345.43 ± 13^{abA}
7	4.62 ± 0.13^{bcA}	2.50 ± 0.20^{cdB}	40.73 ± 6.6^{A}	16.91 ± 4.6^{abB}
8	5.04 ± 0.06^{abA}	3.04 ± 0.31^{abB}	41.69 ± 1.6^{A}	27.25 ± 2.7^{bB}
9	5.27 ± 0.10^{aA}	3.46 ± 0.09^{aB}	42.95 ± 3.1^{A}	27.25 ±1 .5^{bB}

Values are presented as mean ± standard deviation (n = 3);
A, B Values with different letter superscripts within row marks significant difference between muscle portions at p < 0.05;
a, b…Values with different letter superscripts within the column marks significant difference between the storage time at $p < 0.05$.

The a* and b* values can also be used to derive the color (hue angle) and saturation (chroma) of the meat samples (Table 3). Generally, the color of rabbit meat intensified significantly with time, regardless of muscle portion. The chroma values obtained in this study are comparable to similar studies on rabbit meat. Kozioł et al. (2015) monitored the chroma and hue values of rabbit meat stored at chilled temperature for 24 h. The chroma values increased from 4.29 to 7.62. The chroma values also differed significantly in both muscles, which was also found by Koné et al. (2019) and Wang et al. (2016) in rabbit meat. In both studies, *longissimus lumborum* has higher chroma values than *biceps femoris*, indicating that *longissimus lumborum* has a more intense color than the latter.

Total Myoglobin Content

Meat color is dependent on the pigments present in meat. Myoglobin and hemoglobin are the pigments responsible for the red color in meat. Myoglobin comprises the majority of pigment in the muscle. At the same time, hemoglobin contributes pigment in meat to a smaller content brought by residual blood in the muscle and internal organs (Valenzuela et al. 2011). Table 4 shows the total myoglobin content of rabbit meat. Results of this study revealed that the total myoglobin content of rabbit meat remains constant within storage time ranging from 1.37 to 1.92 mg g^{-1} and 1.96 to 3.03 mg g^{-1} for the hind leg and the saddle, respectively.

Table 4. Effect of ambient temperature on the myoglobin concentration and lipid oxidation of rabbit meat during storage.

Storage Time (h)	Myoglobin Concentration, mg g^{-1} Meat		Lipid Oxidation, mg MDA kg^{-1} Meat	
	Hind Leg	Saddle	Hind Leg	Saddle
Initial	1.37 ± 0.15	1.96 ± 0.69	0.04 ± 0.04	0.09 ± 0.01
1	1.51 ± 0.67	2.30 ± 1.08	0.05 ± 0.05	0.07 ± 0.02
2	1.65 ± 0.41	2.00 ± 0.69	0.04 ± 0.01	0.15 ± 0.11
3	1.61 ± 0.19	2.20 ± 0.73	0.06 ± 0.02	0.11 ± 0.12
4	1.63 ± 0.52	2.46 ± 1.16	0.08 ± 0.04	0.14 ± 0.10
5	1.67 ± 0.64	3.03 ± 1.17	0.07 ± 0.02^{B}	0.18 ± 0.05^{A}
6	1.67 ± 0.88	2.45 ± 0.76	0.08 ± 0.02^{B}	0.33 ± 0.07^{A}
7	1.70 ± 0.65	2.43 ± 0.98	0.08 ± 0.02	0.27 ± 0.16
8	1.92 ± 1.06	2.76 ± 1.32	0.08 ± 0.01^{B}	0.34 ± 0.14^{A}
9	1.87 ± 1.21	2.65 ± 1.42	0.09 ± 0.01	0.08 ± 0.02

Values are presented as mean ± standard deviation (n = 3);
A, B Values with different letter superscripts within row marks significant difference between muscle portions at $p < 0.05$.

The color of meat is determined by the form of myoglobin present in the muscle. The total myoglobin content of rabbit meat in this study is expressed as the sum of all its three forms namely: deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb). The proportions of each vary depending on the presence of oxygen and iron state. When there is no oxygen, the DMb form predominates, and the meat appears purple. Once exposed to oxygen, the meat appears bright red and is in the OMb form. However, if the meat is oxidized, the MMb form occurs, which causes the meat to turn brown and is typically an indicator of the end of meat freshness. As a result, previous researchers have focused on studying MMb instead of total myoglobin content as an indicator of meat freshness. MMb usually appears in higher amounts during the latter stages of meat storage.

According to previous studies, the metmyoglobin content of rabbit meat is affected by the storage temperature and muscle portions. Wang et al. (2021) found a variation in the metmyoglobin percentage of rabbit meat stored at different retail storage temperatures (8ºC, 3ºC, and -1ºC). The metmyoglobin percentage of rabbit meat *longissimus thoracis et lumborum* increased significantly with time, but changes were kept at a minimum at lower temperatures. Hence, this indicates that the retail display time of rabbit meat can be extended when meat is stored at a lower temperature. In the same manner, reducing the storage temperature to around 3 – 5ºC can retard the discoloration in rabbit meat by delaying the myoglobin oxidation.

On the other hand, higher myoglobin content was present in meat with higher red muscle fibers. *Biceps femoris* has higher red muscle fiber (*α*R and *ß*R), which ranges from 45.38 – 62.67% (Krunt et al. 2021). Moreover, muscles engaged in extensive movement activities, like hind legs, have greater myoglobin content due to an increasing number of mitochondria than those engaged in support, like the loin. However, in this study, myoglobin content did not vary within storage time at ambient conditions and muscle portions.

The constant values of total myoglobin within storage and between muscle portions might be because the myoglobin content measured in the current study accounts for all its forms. The individual proportions—DMb, OMb, and MMb—were not measured. Based on the values obtained at every sampling point, myoglobin concentration in rabbit meat is constant, as no significant differences exist over time. Thus, this implies that myoglobin is reasonably stable. Metmyoglobin is stable at temperatures less than 55ºC and can tolerate pH up to 5.6 (Zhu and Brewer 2002). In this study, the lowest pH attained by the meat samples was around 5.6. Thus, this explains the consistent values of myoglobin across time.

The results of this study provides a range of values from the total myoglobin content of rabbit meat during storage. The total myoglobin content of rabbit meat is 1.37 – 3.03 mg g^{-1}. Compared with similar livestock meats, rabbit meat has comparable myoglobin content with pork (2 mg g^{-1}), higher than chicken (0.31 mg g^{-1} for breast and 1.17 mg g^{-1} for leg/thigh), and inferior to lamb (6 mg g^{-1}) and beef (8 mg g^{-1}) (Kranen et al. 1998; Mancini and Hunt 2005).

Lipid Oxidation

The degradation of lipids through oxidation produces substances that affect the nutritional and sensory qualities of meat. This makes it an important factor to monitor meat quality during storage. Lipid oxidation occurs when unsaturated fatty acids in meat react with oxygen in the air. This process begins shortly after slaughter and continues until the availability of pro-oxidants like iron and copper depletes (Amaral et al. 2018). Malondialdehyde (MDA) is a byproduct of lipid oxidation that reacts with thiobarbituric acid (TBA) to produce a color adduct that can be measured spectrophotometrically. The changes in lipid oxidation can then be expressed as mg MDA kg^{-1} meat. Rabbit meat has high proportions of unsaturated fatty acids, which makes it more susceptible to lipid oxidation (Wood et al. 2008; Rasinska et al. 2018).

Results revealed that significantly higher oxidation was observed in the saddle muscle than in the hind leg portion of the rabbit. Lipid oxidation was found to be highly correlated to metmyoglobin (Baron and Andersen 2002; Wang et al. 2021). The higher myoglobin values (Table 4) of the saddle over the hind leg muscle could be the reason for this result. Moreover, the *longissimus lumborum* of rabbits was found to have higher proportions of white and fast-twitching muscle fibers, which are known to be more susceptible to early post-mortem proteolytic degradation than red muscle fibers (Joo et al. 2013). As a result of proteolytic degradation in meat, microorganisms proliferate due to more significant amounts of readily digestible nutrients. This will also result in the alteration of oxygen in the meat, hence promoting the oxidation of both myoglobin and lipids. Other factors such as breed (Mugnai et al. 2008), diet of the animal (Dalle Zotte 2014), and meat pH could also affect the rate of oxidation in different muscle parts (Tichivangana and Morrissey 1985).

The concentration of malondialdehyde (MDA) in rabbit meats obtained in this study ranges from 0.04 to 0.34 mg MDA kg^{-1} (Table 4). These values are lower than what is reflected in the literature. Tůmová et al. (2014) reported higher changes in the mg MDA of rabbit hind leg meat (0.64 – 5.22 mg MDA kg^{-1} meat) compared to this study. Meat samples had no significant differences in their MDA values across storage time. This

result is congruent with that reported by Morshdy et al. (2021), where a significant change in MDA values was only observed during the 6th d of storage of rabbit meat at 3 ± 1ºC. Values obtained by Morshdy et al. (2021) were also lower compared to Tůmová et al. (2014), which ranged from 0.05 to 1.34 mg MDA kg^{-1} meat.

It is also important to note that the MDA values of the saddle muscle increased over time but abruptly decreased by the end of the storage period (Table 4). This decrease in lipid oxidation can be attributed to the loss of MDA due to its volatile nature, as well as the depletion of substrate for lipid oxidation reaction. During lipid oxidation, hydroperoxides are formed, which subsequently decompose into secondary products such as alkanes, alcohols, ketones, and aldehydes (Domínguez et al. 2019). Malondialdehyde is the most significant aldehyde produced in lipid oxidation, and it strongly affects the aroma and degradation of the product. Due to its volatile nature and fast oxidation rate, some malondialdehyde can be lost before quantification, resulting in a decrease in the MDA values. Additionally, the substrate for lipid oxidation, PUFAs, could be depleted, resulting in no further product formation.

A value of 1 mg MDA kg^{-1} meat is the arbitrary threshold for the off-flavors to be perceived and associated with oxidative rancidity in pork (McKenna et al. 2005), and 0.9 mg MDA kg^{-1} meat and 5 mg MDA kg^{-1} meat for chicken and beef, respectively. The TBARS value of rabbit meat detected in the study was below the indicated threshold. However, observable off-odors were observed at the latter stage of storage during experiments. This could mean that the TBARS value obtained in this study may indicate an underestimation of the actual MDA formed in the meat. One factor that may have affected this result could be due to sample preparation before analysis. Meat samples were minced before the addition of the extracting solvent. Hence, exposure of the meat samples to oxygen before analysis may already led to the oxidation of some lipids present that were not measured in the study.

Microbiological Quality

Microbiological parameters such as aerobic plate count (APC) and total coliform count (TCC) are important quality indicators in meat, the levels of which relate to the hygienic slaughtering and handling of meat before sale and dictate the meat's shelf-life. Despite rabbit meat being a recent addition to the meat sources for Filipinos, there needs to be more information on the microbiological changes that occur at different storage conditions. Previous research regarding the microbial quality of rabbit meat was conducted in other countries with variations in the breed and environmental conditions in which the rabbit is reared, slaughtered, and stored. Thus, this would be the first study of the microbiological changes in rabbit meat in the Philippine setting. The results of this study revealed significant changes in microbial counts across time in both muscle portions.

The APC of a sample serves as an indicator of the overall level of contamination in meat. On the other hand, TCC provides the sanitary conditions in which the meat is slaughtered and held. The initial APC of the hind leg and saddle are 4.69 ± 0.17 log CFU g^{-1} and 4.29 ± 0.30 log CFU g^{-1}, respectively (Table 5). These initial values of newly slaughtered meat agree with the initial microbial load of freshly slaughtered meat. Similar results were obtained by Rodríguez-Calleja et al. (2004), who reported APC counts of rabbit carcass dressed in small (4.01 ± 0.48 log CFU g^{-1}) and large abattoir (4.96 ± 0.90 log CFU g^{-1}). On the other hand, the TCC obtained in this study is higher compared to Rodríguez-Calleja et al. (2004), which only ranges from 1.55 to 2.02 log CFU g^{-1}.

Table 5. Effect of ambient temperature on the aerobic plate count (APC) and total coliform count (TCC) of rabbit meat during storage.

Storage Time (h)	Aerobic Plate Count, Log CFU g^{1}		Total Coliform Count, Log CFU g^{-1}	
	Hind Leg	Saddle	Hind Leg	Saddle
Initial	4.69 ± 0.17^{c}	4.29 ± 0.30^{d}	2.40 ± 0.00^{f}	2.40 ± 0.00^{e}
1	4.36 ± 0.38^{c}	4.02 ± 0.27^{d}	2.40 ± 0.00^{f}	2.40 ± 0.00^{e}
2	4.45 ± 0.35^{c}	4.10 ± 0.11^{d}	2.73 ± 0.00^{f}	2.40 ± 0.00^{e}
3	4.73 ± 0.40^{c}	4.67 ± 0.43^{dc}	3.13 ± 0.07^{fed}	3.13 ± 0.08^{ed}
4	5.59 ± 0.31^{cb}	4.90 ± 0.34^{cd}	3.73 ± 0.10^{ed}	3.53 ± 0.10^{d}
5	5.87 ± 0.34^{cb}	5.62 ± 0.35^{cb}	3.88 ± 0.18^{d}	4.38 ± 0.29^{c}
6	6.29 ± 0.40^{cb}	6.34 ± 0.17^{b}	4.21 ± 0.49^{dc}	4.91 ± 0.05^{cb}
7	7.26 ± 0.18^{ba}	7.46 ± 0.22^{a}	5.09 ± 0.16^{cb}	5.37 ± 0.30^{b}
8	7.64 ± 0.41^{ba}	7.54 ± 0.34^{a}	5.83 ± 0.21^{ba}	6.25 ± 0.24^{a}
9	8.47 ± 0.04^{a}	8.44 ± 0.09^{a}	6.72 ± 0.37^{a}	7.04 ± 0.10^{a}

Values are presented as mean±standard deviation (n = 3);
a, b…Values with different letter superscripts within the column marks significant difference between the storage time at $p < 0.05$.

The presence of high levels of coliforms in meat indicates fecal contamination due to poor hygiene and unsanitary conditions during slaughtering and handling processes. Coliform bacteria include both fecal (*Escherichia coli*) and non-fecal species (e.g., *Klebsiella* and *Enterobacter*). Raw meat becomes unacceptable when it reaches the 5 x 10^6 (6.70 log) CFU g^{-1} limit for APC and of 5 x 10^2 (2.70 log, Raw, Meat, RTE) and 5 x 10^3 (3.70 log, Poultry) CFU g^{-1} limit for the TCC (BAFS 2023). In this study, the APC and TCC limits were reached after 6 h and 4 h of storage, respectively. With these conditions, meat is considered spoiled and must not be sold at any cost.

In the Philippines, raw meat is sold in open-air local wet markets without appropriate temperature control. It has been known that microorganisms are ubiquitous. Thus, exposing

the meat to open air provides an avenue for microorganisms to grow. Based on the DA-NMIS (2012) guidelines, meat sellers are only allowed to display newly slaughtered meat for 8 h, after which the meat is considered spoiled and unsaleable. However, this guideline may not apply to all types of meat. For instance, pork exceeds the microbiological limits after 5 h of storage at ambient temperature (Pel et al. 2017), and chicken after 3 and 5 h (Manalo and Gabriel 2020). In this study, meat samples were stored in polystyrene trays and covered with polyvinylchloride plastic film, yet rabbit meat samples are considered spoiled between 4 – 6 h of storage at ambient conditions. With these results, it may be essential to revisit the existing guidelines considering the safety of the consuming public.

It is worth noting that the APC in rabbit meat shows no significant differences ($p < 0.05$) as storage time increases. This could be due to the overpopulation of microorganisms, resulting in a depletion of available nutrients in the meat. This lack of nutrients inhibits further growth of the microbial load, known as the stationary phase. Pel et al. (2017) observed the same phenomenon in their study, where they found that after the APC count of fresh pork reached 7 log CFU g^{-1}, further exposure of meat no longer resulted in differences in the microbial count of fresh pork. Furthermore, there is no significant difference in the microbial population between the two muscle portions, which supports the study of Mahmoud et al. (2022). The latter found no differences in the APC, yeast, and mold count and Enterobacteriaceae in four different cuts of rabbit meat such as shoulder, ribs, loin, and thigh.

Correlation of All Parameters Measured

Kendall's Tau correlation was performed to determine the relationship of all the parameters measured in this study. Generally, there was a strong positive correlation between APC and TCC (Tables 6 and 7). These two parameters are expected to be correlated as both methods measure microbial growth of rabbit meat over time. TCC has lower microbial counts than APC because it uses selective media only to capture lactose-fermenting microorganisms. Nonetheless, both exhibit a significantly increasing trend during storage. A positive correlation was also determined by Kim and Yim (2016) in the APC and coliform count (r = 0.517, $p < 0.001$) of beef, pork, and chicken.

The APC and TCC of rabbit meat exhibited a negative correlation with pH. This means that pH decreased as microbial counts (ACC and TCC) increased. This decline in pH is attributable to the growing population of acid-producing microorganisms, e.g., lactic acid bacteria and coliforms in rabbit

Table 6. Correlation coefficients for the measured parameters of rabbit meat hind leg.

Parameters	Aerobic Plate Count	Total Coliform Count	pH	Lipid Oxidation	Myoglobin Concentration	L*	a*	b*
Aerobic plate count	-							
Total coliform count	0.790*	-						
pH	-0.763*	-0.835*	-					
Lipid oxidation	0.357*	0.394*	-0.394*	-				
Myoglobin concentration	0.101	0.045	-0.095	0.249	-			
L*	0.755*	0.722*	-0.729*	0.388*	0.256*	-		
a*	0.672*	0.750*	-0.780*	0.300*	0.039	0.601*	-	
b*	0.683*	0.761*	-0.749*	0.334*	0.152	0.746*	0.723*	-

*Values are significant at $p < 0.05$

Table 7. Correlation coefficients for the measured parameters of rabbit meat saddle.

Parameters	Aerobic Plate Count	Total Coliform Count	pH	Lipid Oxidation	Myoglobin Concentration	L*	a*	b*
Aerobic plate count	-							
Total coliform count	0.851*	-						
pH	-0.743*	-0.787*	-					
Lipid oxidation	0.274*	0.303*	-0.215	-				
Myoglobin concentration	0.159	0.173	-0.141	0.232	-			
L*	0.718*	0.777*	-0.681*	0.313*	0.377*	-		
a*	0.773*	0.876*	-0.737*	0.272*	0.157	0.772*	-	
b*	0.755*	0.878*	-0.804*	0.267*	0.17	0.776*	0.841*	-

*Values are significant at $p < 0.05$

meat. The organic acids produced by these microorganisms caused the pH of the meat to decrease. A similar result was observed in a study on beef stored at 4°C for 2 wk, where a negative correlation existed between TCC and pH (Kunová et al. 2012). On the other hand, no correlation between pH and APC was found in a study on pork and chicken that were stored at ambient temperature (Manalo and Gabriel 2020). The pH values were also not significant across the time of storage. The meat type and initial microflora concentration could be the reason for this difference. It should be noted that storage conditions play a crucial role in demonstrating the relationship between microbial counts and pH since different microbial species may proliferate at different temperatures. In the same study by Kunová et al. (2012), a positive correlation between pH and TCC was observed after 1 wk of storage at 4°C. However, prolonged storage of the meat resulted in a shift in correlation between the parameters.

Microbial growth also affects meat color. This is demonstrated by a positive correlation between APC and TCC with color a* (redness) and b* (yellowness) values in all treatments. Canonical analysis of Wang et al. (2021) shows that a* and b* values are associated with APC. The b* value was said to be positively influenced by APC, i.e., an increased microbial growth will correspond to an increase in the yellowness of the meat. The a* values, on the other hand, have a negative correlation with the microbial count. When microorganisms are present in the meat, they lower the oxygen concentration which eads to changes in the color of the meat pigment myoglobin (Walker 1980). As a result, the L*, a*, and b* values of the meat are also affected, as demonstrated in this study.

Research findings reveal that the color of meat is determined by the amount of myoglobin present and the proportions of its three forms. However, this study established a weak to moderate positive correlation between total Mb and L* (lightness) in all treatments. This is contrary to the negative correlation observed by Hernández et al. (2016) and Wang et al. (2021) between Mb and L*, i.e., when L* is lower, meat is expected to be darker, hence containing higher pigment. This result is supported by several studies showing the L* values of beef (37 – 40) (Hernández et al. 2016) being the lowest than pork (46.4) (Holmer et al. 2009) and chicken (51.6) (Allen et al. 1997). Conversely, increased L* values with high values of a* and b* also indicate darker meat (Kozioł et al. 2015) and increased %MetMb (Zhang et al. 2019), as observed in this study. This increasing trend of L* is also affected by pH negatively, i.e., lower pH will have higher L* because of the reduction in the depth of penetration by light and the increase of reflectance. This result is congruent with the findings of Jankowiak et al. (2021) on pork.

All color values also displayed a positive correlation against lipid oxidation in rabbit meat stored at ambient temperature. High temperature hastens lipid oxidation in meat. Among the three color values, b* is the most associated value that suggests oxidation in meat. Similar results of Wang et al. (2021) and Jankowiak et al. (2021) demonstrated a positive correlation between b* values and TBARS in rabbit meat and pork, respectively. On the other hand, there is a weak correlation between lipid oxidation and microbial growth. Lipids are a known source of nutrients for some microorganisms. Consequently, lipids are hydrolyzed and degraded into simpler compounds, which make them susceptible to oxidation. Hence, increased microbial growth may lead to faster oxidation of lipids in meat (Branen n.d.).

CONCLUSION AND RECOMMENDATION

The changes in rabbit meat during storage at ambient temperature were established in this study. The pH of rabbit meat decreased over time, irrespective of its muscle portions. The decline in pH is caused by the accumulation of acids produced by spoilage microorganisms. Additionally, the color values of rabbit meat tended to increase with time, which is an indication of meat discoloration caused by both chemical and microbiological factors. The total myoglobin content of rabbit meat remained constant within the storage period, owing to the stability of myoglobin relative to the pH attained by the meat. Similarly, no significant change was observed in lipid oxidation during storage, but significantly higher oxidation was observed in the saddle muscle at the latter part of the storage. This is likely due to the greater stability of the myoglobin content of the saddle muscle than the hind leg since myoglobin concentration was a significant precursor in lipid oxidation reactions. The microbial data of rabbit meat indicated that both rabbit muscles exceeded the microbiological limits for TCC and APC after 4 and 6 hrs of storage, respectively. This study highlights that, following slaughter, rabbit meat must be subjected to ambient storage conditions for the shortest possible duration. Prolonged storage under these conditions may result in the spoilage of the meat rendering it unsuitable for human consumption. Hence, it is recommended that careful consideration be given to the duration of ambient storage when handling rabbit meat, to ensure that the quality and safety of the meat are upheld.

For further studies, it is also recommended to determine the changes in other portions of the rabbit carcass, such as the foreleg and the thorax. A real-time analysis of lipid oxidation and myoglobin content is also suggested to improve the results of the study. Moreover, aside from aerobic plate count and total coliform count, which estimate the microbial population of the meat and signify hygienic practices, it is also essential to determine the prevalence of some pathogenic microorganisms in rabbit meat, such as *Escherichia coli* O157:H7 and Salmonella.

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