

NIVERSIDAD



Revista Científica, FCV-LUZ / Vol. XXXII, rcfcv-e32121, 1-6

Profile of Nitrogenous compounds and Bacterial proliferation in Rabbit meat stored cold with three types of packaging

Perfil de compuestos nitrogenados y proliferación bacteriana en carne de conejo almacenada en frio con tres tipos de empaques

Technical note

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ABSTRACT

Meat is an excellent medium for bacterial growth due to its high water and nutrient content. The nitrogenous compounds (NC) are derived through decarboxylation of amino acids due to microbial enzymes. The objective of this study was to evaluate the concentration of 3 NC and the proliferation of some microorganisms in rabbit meat with three treatments(T), classified by three types of packaging for 21 days(d) in rabbit meat stored cold. The meat samples were obtained of the Longissimus thoracis et lumborum muscle. Each sample was divided and two groups were formed. The first group was used to measure the physicochemical characteristics of the meat, and the second group was used to quantify NC and bacterial isolation. The pH in the meat decreased from 0 to 21 d in the three T. The brightness (L*) decreased (P<0.05), while the variables a* and b* increased (P<0.05) to 21 d for all groups. Histamine and cadaverine remained low and were similar in the three T (P>0.05). Putrescine (PU) increased (P<0.05) from 7 to 21 d in the Control-Plastic (CP) and Semi-permeable plastic film (SP) groups vs. Vacuum packing (VP). The Enterobacteriaceae remained constant throughout the experimental period in the three T, compared to the aerobic mesophilic, which was higher (P<0.05) until 21 d of the evaluation in CP and SP. The type of packaging and cooling time influenced the concentration of NC. The VP had the lowest level of PU and mesophilic bacteria until 21 d of storage.

Key words: Bacteria contamination; meat; packaging; rabbit

RESUMEN

La carne es un excelente medio para el crecimiento bacteriano debido a su alto contenido de agua y nutrientes. Los compuestos nitrogenados (NC) se obtienen a través de la descarboxilación de aminoácidos debido a las enzimas bacterianas. El objetivo de este estudio fue evaluar la concentración de 3 NC y la proliferación de algunos microorganismos en carne de conejo con tres tratamientos (T), clasificados por los tipos de empaques almacenada en frío durante 21 días (d). Las muestras de carne se obtuvieron del músculo Longissimus thoracis et lumborum. Cada muestra se dividió y se formaron dos grupos. El primer grupo se utilizó para medir las características fisicoquímicas de la carne, y el segundo grupo se utilizó para cuantificar los CN y el aislamiento bacteriano. El pH en la carne disminuyó de 0 a 21 d en los tres T. La luminosidad (L*) disminuyó (P<0,05), mientras que las variables a* y b* aumentaron (P<0,05) a los 21 d para todos los T. La histamina y cadaverina se mantuvieron bajas y fueron similares en los tres T (P>0,05). La putrecina (PU) aumentó (P<0,05) de 7 a 21 d en los grupos Control-plástico (CP) y Película plástica semipermeable (SP) vs. Empaque al vacío (VP). Las bacterias Enterobacteraceae se mantuvieron constantes durante todo el período experimental en los tres T, en comparación con las bacterias aerobias mesófilas, las cuales fueron superiores (P<0,05) hasta los 21 d de evaluación en CP y SP. El tipo de empaque y el tiempo de enfriamiento influyeron en la concentración de NC. El VP tuvo el nivel más bajo de PU y bacterias mesófilas hasta los 21 d de almacenamiento.

Palabras clave: Contaminación bacteriana; carne; empaque; conejo



INTRODUCTION

Meat is one of the most perishable foods due to its high-water content and available nutrients. Still, when there are physical changes in color, smell, texture, oxidation, and growth of microorganisms, there is a rejection of meat from the consumers. A bacterial load greater than 10^7 colony forming units square centimeter $^{-1}(CFU \cdot cm^{-2})$ in the meat packets causes a bad smell, and an amount of 10^9 CFU $\cdot cm^{-2}$ the smell was putrid, causing decarboxylation of the free amino acids [8]. During the decomposition of the meat, there is a formation and accumulation of nitrogenous compounds (NC) [22, 31]. The quantification of the NC in the meat indicates the beginning of the microbial activity and its deterioration in nutritional value [21, 22].

There are two standard packaging systems; these are the conventional polystyrene foam tray and vacuum packaging [7]. Specifically, there is no information on rabbit (*Oryctolagus cuniculus*) meat quality over shelf life. The objective of the study was to identify NC production, microbial contamination, and some quality parameters in rabbit meat, using three types of packaging at different storage times.

MATERIAL AND METHODS

Study place

The animals were obtained from the rabbit farm of the Chapingo University, Mexico, located at 19°29' North and 98°54' West [17]. Physicochemical analyses of the meat and the identification of bacteria were carried out in the Facultad de Estudios Superiores, Cuautitlán of the National University.

Selection and slaughter of rabbits

Sixty adult male rabbits, New Zealand breed, were divided into three experimental treatments (T), homogeneous in weight (2.35 ± 0.25 kilograms (kg). Each T was housed in three-place cages (30×42 centimeters -cm-, height 30 cm). All the animals were slaughtered not longer than 2 minutes (min) after each animal was removed from its cage. The procedure was bled by cutting the jugular vein and the carotid artery (less than 30 seconds -s -) and then the skin, genitals, urinary bladder, gastrointestinal tract and distal parts of legs were removed [14].

Obtaining samples

Previously, a refrigerator (Imbera, VR19, Koblens, USA) was assigned to preserve the meat samples, and it was disinfected with 10% of the nitric acid solution and washed with distilled and deionized water. Then, 30 grams (g) of the *Longissimus thoracis et lumborum* muscle of each animal was obtained. Each sample was divided into six portions of 5 g and two groups were formed (3 sub-samples in each group). The first group was used to measure the physicochemical characteristics of the meat, and the second group was used to quantify NC and bacterial isolation.

All samples were identified inside plastic bags and refrigerated $(4^{\circ}C)$ at times zero (less than 12 cooling hours -h-: 0, 7, 14, and 21 days -d-, 45 samples for each T-time). Subsequently, the samples stored for each time were randomly divided into 15 samples for each type of packaging, classified into the following Ts:

 Control-plastic (CP), samples covered with a transparent plastic bag (polyethylene). 15×25 cm. Caliber: 18 milimicra (μm).

- Polyethylene tray with semi-permeable plastic film (SP): Unicel tray (Reyma[®]) with 11 cm diameter and a flexible food-grade film (12 μm).
- Vacuum packing (VP): Vacuum bags of 15×20 cm and 90 μ-2.8 mililiters⁻¹-mL- (Torrey[®] packer model-Evd20); the vacuum time was 40 s and the sealing of 2 s.

Physical measurements of meat

The pH and colorimeter electrodes were previously disinfected between each sample with a 10\% nitric acid solution and washed with deionized water.

Meat pH

A portable potentiometer (HANNA model-HI99163, USA) was used. The electrode was pressed moderately on the surface of each sample, and the pH reading was recorded for four consecutive times.

Meat color

A Hunter Lab portable colorimeter (CR-410, Konica-Minolta, Inc. Japan) was used to measure the variables L* (luminosity), a* (red-green) and b* (yellow-blue). It was calibrated with the tile to the reference coordinates: L* = 94.7, a* = 0.3130, b* = 0.3191); subsequently, three points were measured on the surface area of the meat sample.

Bacteriological tests

Samples of 5 g of meat were mixed with 45 mL of sterile physiological saline; serial dilutions were prepared up to 1:1000. Two agar media Standard (Bioxon[®]) was used for the quantify *Enterobacteriaceae* (Violet Red Bile Glucose Agar: MH581) and total aerobic mesophilic (Plate Count Agar: ICMSF – 2000). The incubation was carried out from 24 to 48 h at 35° C, in a Felisa[®] equipment (Felisa: FE-500, Feligneo, México). The volume spread calculated the number of colonies that arose on a pour plate at the end of the designed time.

Quantification of nitrogenous compounds

The samples were removed from the assigned packing and they were macerated to measure NC through a 3-phase procedure [22]:

First phase. Preparation of solution and individual standards of the NC: In each tube, putrescine (PU), cadaverine (CA), histamine (HI) standards (Sulpenco, Merck, USA) were weighed to have a concentration of 10 milligrams mL^{-1} (mg·mL⁻¹). Each standard was diluted in 1 mL of hydrochloric acid and homogenized (base solution).

Second phase. Extraction: 5 g of each meat sample was weighed into test tubes, and 5 mL of perchloric acid (6%) was added, homogenizing in the vortex (Scientific Industries, SI-0236, USA) and allowed to stand for 1 h in refrigeration. All samples were centrifuged (Universal 320/320R Hettich®, México) at 4950G-force (10 min at 4°C), and the solutions were filtered, adding 1 mL of 2 Molar (M) NaOH. The pH was maintained at 6 and the tubes were kept in ice water for 20 min. Derivatization: All tubes were removed from the cold water, and 20 microlliters (μ L) of benzoyl chloride was added and homogenized. They were left at rest for a further 20 min and 2 mL of 5 M sodium chloride was added. Then, the ether sample was evaporated with a flow of nitrogen (10 min) and 500 μ L of Milli Q water and 500 μ L of acetonitrile were added and vortexed.

High-performance liquid chromatography

Third phase: The filtered sample (1 mL) was injected into the Highperformance liquid chromatography (HPLC) with a diode detector array (Model 1100 Horse Power(HP) Agilent Technologies. Wilmington, USA). An elution gradient program was used with a 50:50 mixture, acetonitrile as solvent A and purified ultrapure water as solvent B. The flow rate used was 1 mL·min⁻¹. The temperature of the column was 40°C, and the effluent from the column was analyzed at 254 nanometers.

Statistical analysis

All rabbits were randomly distributed with a 4×3 factorial arrangement. The normal distribution in the number of bacterial colonies was determined with the Kolmogorov-Smirnov test. All the variables were analyzed with a PROC MIXED design [28] and the mean comparison was made with the PDIFF test, using the statistical package [28].

RESULTS AND DISCUSSION

pH and bacterial count

TABLE I shows all results. The pH in the CP, SP, and VP Ts decreased 0.74, 0.85, and 1.08 units (u) from 0 to 21 d, respectively. There were no differences (P>0.05) in pH between the three Ts at storage times of 0, 7, and 14 d, the value of pH=6.7 was within the range reported by other authors [4, 23]. Except for 21 d, the pH of the CP T was higher (6.28, P<0.05) and decreased 0.21 and 0.45 u for the SP and VP Ts (P<0.05), respectively [9]. Possibly, this effect is due to the accumulation of lactic acid, which could cause protein denaturation and water retention decreased [15]. The lower concentration of pH in the VPT was possibly due to the higher content of lactic acid accumulated in this type of packaging and a lower bacterial proliferation of *aerobic mesophiles* and Enterobacteriaceae (TABLE I), due to the vacuum process (EVAC-8-RHI, Inox, NH_Rhino, China). Other authors report that contamination with bacteria and some fungi could induce the formation of alkaline compounds [2,16], causing the decomposition of meat and increase of pH and ammonia [4, 30].

Color

The L* value decreased (P<0.05) 2.52, 3.99, 4.96 u from 0 to 21 d for the CP, SP and VP Ts (TABLE I). Contrarily the values of a* and b* increased (P<0.05) 2.85, 3.07, 3.27, and 5.96, 7.77, 4.06 u from 0 to 21 d for the CP, SP and VP Ts, respectively. The L* and a* indices between the CP, SP and VP Ts did not show significant differences (P>0.05) from the times 0 to 21 d; except fora decrease of 2.4 u in L* of SP T compared with VP T. The b* index was similar in all Ts at 0 d (P>0.05), although from 7 to 21 d of the SP T (average: 11.7) was increased 1.63 u, while the VP T (average 7.25) decreased 2.82 u (P<0.05), both compared to the CP T (average 10.07).

The L* decreased with the cooling time, but the index a* and b* increased (P<0.05) in the three types of packages evaluated. There is no published data on rabbit meat, but color values were similar to bovine meat [10, 25]. L* value was influenced by the concentration of reduced myoglobin, oxymyoglobin, and metmyoglobin [13] and the index a* and b* increase with the maturity time of meat due to the greater passage of light in the meat tissue. Maybe it was associated with the opening of the packages, and the meat had contact with oxygen due to which myoglobin was transformed into oxymyoglobin and, it was intensifying the brightness and red color [24].

| TABLE I |
|---|
| Physico-chemical variables and bacterial count (mean ± sd) in three |
| of packaging at different days of refrigeration in rabbit meat |
| (muscle Longissimus thoracis et lumborum) |

| | Refrigeration days | | | | |
|-----------------------------------|--------------------|-----------------|-----------------|-----------------|--|
| | 0 | 7 | 14 | 21 | |
| Control Plastic (CP) | | | | | |
| рН | 6.65 ± 0.05cA | 5.70 ± 0.01aA | 5.74 ± 0.05aA | 6.28 ± 0.14bC | |
| Meat col | our | | | | |
| L* | 58.97 ± 1.03cA | 57.60 ± 0.48bcA | 56.44 ± 0.47abA | 55.30 ± 0.53aAB | |
| a* | 14.62 ± 0.86aA | 17.34 ± 0.39bA | 17.16 ± 0.46bA | 17.93 ± 0.85bA | |
| b* | 4.11 ± 0.80aA | 9.57 ± 0.23bB | 10.54 ± 0.27bB | 10.11 ± 0.31bB | |
| Semi-permeable (SP) plastic film) | | | | | |
| рН | 6.69 ± 0.05cA | 5.74 ± 0.04aA | 5.71 ± 0.03aA | 6.07 ± 0.11bB | |
| Meat col | or | | | | |
| L* | 60.05 ±1.26cA | 57.55 ± 0.53bA | 56.98 ±0.66bA | 53.64 ± 0.73aA | |
| a* | 15.06 ± 1.14aA | 17.97 ± 0.43bA | 17.96 ± 0.42bA | 18.47 ± 0.47bA | |
| b* | 3.98 ± 0.88aA | 11.45 ± 0.35bC | 11.53 ± 0.27bB | 12.29 ± 0.39bC | |
| Vacuum packing (VP) | | | | | |
| рН | 6.70 ± 0.05bA | 5.55 ± 0.03aA | 5.58 ± 0.03aA | 5.73 ± 0.03aA | |
| Meat col | or | | | | |
| L* | 60.39 ± 0.78cA | 58.20 ± 0.51bA | 56.50 ± 0.78abA | 56.08 ± 0.45aBC | |
| a* | 13.71 ± 0.67aA | 17.00 ± 0.36bA | 16.95 ± 0.37bA | 17.01 ± 0.29bA | |
| b* | 3.19 ± 0.41aA | 7.06 ± 0.21bA | 7.45 ± 0.21bA | 7.26 ± 0.16bA | |

CP: Polyethylene plastic bag, SP: Polyethylene tray with semipermeable plastic film, VP: Vacuum packed (vacuum time 40 seg and sealing 2 seg), s.d.: standard deviation, L*: luminosity, a*: index color red-green, b*: index color yellow-blue. a-c: Different lowercase letters in the same rows indicate significant differences between storage days (P<0.05). A-C: Different capital letters in the same column indicate significant differences between packages (P<0.05)

Nitrogenous compounds and bacterial contamination

The PU content did not show significant differences (P> 0.05) between the three T during the first 7 d storage (TABLE II). Then, PU increased drastically (P<0.05) from 7 to 21 d of storage. CA and HI had significant differences (P<0.05) from 14 to 21 d between the SP and VP T. The storage time and the type of packaging mainly influenced the production of PU, and a high level of PU is associated with the proliferation of *Pseudomonas* spp. in aerobic conditions at 37°C[1]. The PU was the main NC formed; its value was similar to another report in chicken(*Gallus gallus familiaris*)(45.2 mg·kg⁻¹[22]) during 17 d of storage. In contrast, the average concentration of CA in the three T was lower (2.54 mg·kg⁻¹) than reported (5.7 mg·kg⁻¹) in chickens at the end of the evaluation. CA and HI at 7 d of storage had a higher concentration than in bovine (*Bos taurus*) meat (1.85 and 2.11 mg·kg⁻¹), stored for 7 d in trays packing with trays of polystyrene [12].

The production of CA in rabbit meat is possibly associated with lysine; this amino acid was the precursor of NC[5]. *Enterobacteriaceae* also induced the highest CA content [6]. However, the number of these bacteria was lower than that reported by other authors [1, 12], which suggests that a smaller amount of *Enterobacteriaceae* is associated



CP: Polyethylene plastic bag, SP: Polyethylene tray with semipermeable plastic film, VP: Vacuum packed (vacuum time 40 seg and sealing 2 seg). s.d.: standard deviation, En: *Enterobacteriaceae*, Mes: *Mesophiles*, Un: Undetermined, a-c: Different lowercase letters in the same rows indicate significant differences between storage days (*P*<0.05), A-C: Different capital letters in the same column indicate significant differences between packages (*P*<0.05)

with a lower amount of CA. On the other hand, the PU content compared to CA was similar to other studies [19, 29]. Also, NC can be formed by the degradation of glutamine, arginine, and agmatine. Arginine is easily converted into agmatine by the decarboxylation of arginine by agmatine-deiminase and transformed to PU [6,18].

Although, HI concentration was greater than the CA [12, 19] and CA concentration was not a risk of intoxication in this study, the permissible CA limit was about 40 mg [18, 27]. The increase of NC was given with the cooling time [1, 20]. In this regard, Vinci and Antonelli [32] evaluated the amount of NC in bovine meat and chicken meat; finding that NC production differs between two types of meat up to 15 d of storage. The proteolytic enzymes easily attack meat chicken, and consequently, there is better availability of amino acid precursors for NC. In the case, rabbit meat has small fibers, but the collagen content of the Longissimus muscle is higher (7.0 mg·g⁻¹) when compared to the collagen of pigs (Sus scrofa domesticus) $(5.9 \text{ mg} \cdot \text{g}^{-1})$, bovines (3.4-5.8 mg·g⁻¹) and chickens (3-4 mg·g⁻¹). The collagen causes greater hardness of the meat and possibly prevents the attack of the proteolytic enzymes, consequently there may be less availability of amino acid precursors for the formation of NC [26]. So, the lower content of NC in rabbit meat can be due to this effect explained and, the activity amino acid-decarboxylase influenced by bacteria [1, 12]. Temperature, oxygen availability, redox potential, and pH[11, 30] are other factors that also participate in the NC formation.

The detection of HI in chicken meat was reported from 11 d of storage [1] and in fish (*Cyprinus carpio*) meat on the 3^{th.} d, with a maximum peak at 12 d of storage [4]. In this study, the three types of packaging showed an increase in HI with storage time. In reference, PU in the CP and SP packages was related to the presence of high oxygen and less carbon dioxide levels, unlike the VP generating selective microbial proliferation [29]. The most common bacteria in VP were aerobic mesophiles and Enterobacteriaceae[1]. The aerobic mesophiles were higher at the end of the evaluation in the CP and SP(\sim 4.7), but the VP T was similar throughout this experiment (TABLE II). It is difficult to have a direct correlation between the counts of microorganisms and NC[6]; although in this study was observed that the PU content and the aerobic mesophiles count were higher in the CP and SP. The results were similar with beef vacuum packed, where the count of aerobic mesophiles [8] and Enterobacteriaceae [3, 8, 9] increased in 14 d of storage. Currently, there is interest in Enterobacteriaceae detection in food samples, because these microorganisms are a public health risk by toxicological effects.

CONCLUSION

The storage time changed the variables of color, pH, NC content, and bacterial count. At the end of the evaluation (21 d), there were differences in conservation time and the packaging type. The number of *Enterobacteriaceae* was not altered by the storage time. The type of packaging and the cooling time influenced the concentration of NC; mainly the VP had the lowest concentration of PU and mesophilic bacteria until 21 d of storage.

Conflict of interest

The authors declare no potential conflicts of interest.

ACKNOWLEDGEMENTS

This research was supported by the Universidad Autónoma Metropolitana Lerma and Colegio of Postgraduados of Mexico.

Ethics Approval

The study was approved and conducted in accordance with the guidelines of the Institutional Animal Care of Colegio de Postgraduados. México. (Approval ID: 09-2019).

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