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Development of microbial spoilage and lipid and protein oxidation in rabbit meat

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ABSTRACT

This experiment aimed to determine microbial spoilage and lipid and protein oxidation during aerobic refrigerated (4 °C) storage of rabbit meat. Forty male New Zealand white rabbits were slaughtered according to the Halal slaughter procedure. The hind limbs were used for microbial analysis while the *Longissimus lumborum* m. was used for determination of lipid and protein oxidation. Bacterial counts generally increased with aging time and the limit for fresh meat (10^8 cfu/g) was reached at d 7 postmortem. Significant differences in malondialdehyde content were observed after 3 d of storage. The thiol concentration significantly decreased with increase in aging time. The band intensities of myosin heavy chain and troponin T significantly reduced with increased refrigerated storage while actin remained relatively stable. This study thus proposes protein oxidation as a potential deteriorative change in refrigerated rabbit meat along with microbial spoilage and lipid oxidation.

1. Introduction

Due to its high digestibility, medium-low juiciness, little coarseness, odor and flavor, low-calories, lowest fatty feeling in the mouth and tenderness, rabbit meat is gaining preference by many meat consumers. Compared to red meats, rabbit meat has lower calories (on average 618 kJ/100 g fresh meat), fat (on average 6.8 g/100 g fresh meat), and cholesterol content (on average 53 mg/100 g fresh meat), iron (1.34 mg/100 g), sodium (47 mg/100 g) and energy (119 kcal/100 g) (Cavani, Petracci, Trocino, & Xiccato, 2009; Mačanga et al., 2011). Rabbit meat fits well the contemporary consumer demand for a low-fat meat with a high degree of unsaturated fatty acids and low cholesterol and sodium levels (Cavani et al., 2009; Hernández & Gondret, 2006).

Aging is one of the most critical factors that influence meat quality. During aging, the process of muscle conversion into meat is accompanied by quantitative changes in several metabolites. Consequently, meat becomes unfit for human consumption as it is considered to be spoiled. Spoilage of raw meat accounts greatly for major annual losses to meat processors and retailers (Nattress, Yost, & Baker, 2001). Bacteria levels between 10^7 and 10^9 cfu/cm^2 during refrigerated storage (Borch, Kant-Muemans, & Blixt, 1996) and TBARS values equal to or greater than 5 mg MDA/kg meat (Insausti et al., 2001) comprise the threshold for detecting fitness for human consumption. Owing to the assumption that lipid oxidation was, together with microbial spoilage, the only causes of food deterioration resulted in ignoring the fact that proteins are targets for reactive oxygen species (ROS) for several decades. The discovery that myofibril proteins are affected by ROS during meat maturation and storage (Martinaud et al., 1997) has prompted studies related to protein oxidation.

Oxidative reactions occur during storage and processing of meat, and meat products, and such processing steps such as mincing, cooking, and salt addition that promote the formation of ROS increase the susceptibility of products to oxidation. Protein oxidation induced by ROS can cause modification of backbones and side chains of proteins, which leads to structural changes at the levels of primary, secondary, and tertiary structures of proteins (Zhang, Xiao, & Ahn, 2013). These structural changes can induce conformational and functional modifications of proteins including protein solubility (Decker, Xiong, Calvert, Crum, & Blanchard, 1993; Nieto, Jongberg, Andersen, & Skibsted, 2013; Xiong, 2000), rehydration properties (Nieto et al., 2013; Xiong, 2000), protein fragmentation and aggregation (Decker et al., 1993; Promeyrat et al., 2011; Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008), protein surface hydrophobicity (Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008; Traore et al., 2012), viscosity, gelation and emulsification (Nieto et al., 2013; Xiong, 2000), and amino acid bioavailability (Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008). It is also thought to negatively impact

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meat color (Filgueras et al., 2010; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a,2004b) and tenderness (Kim, Bødker, & Rosenvold, 2012; Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007; Melody et al., 2004; Rowe et al., 2004a,2004b; Zakrys-Waliwander, O'Sullivan, O'Neill, & Kerry, 2012) and decrease water holding capacity (Decker et al., 1993; Melody et al., 2004).

Unlike red meats, there are a few studies about the shelf life and microbial quality of rabbit meat and the changes it may undergo during storage (Rodríguez-Calleja, García-López, Santos, & Otero, 2005; Rodríguez-Calleja, Isabel, García-López, Santos, & Andrées, 2006; Rodríguez-Calleja, Santos, Otero, & García-López, 2010; Sunki, Annapureddy, & Rao, 1978). Bobbitt (2002) studied the shelf life of rabbit carcasses stored at 4 °C and estimated a shelf life of only 3 d for boxed rabbit carcasses. However, in line with the study of Rodríguez-Calleja et al. (2005), a more recent study by Pereira and Malfeito-Ferreira (in press) has also reported a shelf life of 6–7 d under aerobic refrigerated storage. Lipid oxidation in rabbit meat is limited to the study of Nakyinsige et al. (2014) who reported 0.196 mg MDA/kg meat after 7 d of refrigerated storage at 4 °C while protein oxidation is limited to the studies of Gil et al. (2006) and Prates, Ribeiro, and Correia (2001) who reported degradation of myofibrillar proteins during rabbit meat aging. Thus this study sets out to determine microbial spoilage and lipid and protein oxidation of rabbit muscles during refrigerated storage.

2. Materials and methods

2.1. Ethical note

This study was conducted following the animal ethics guidelines of the Research Policy of Universiti Putra Malaysia.

2.2. Slaughter

A total of 40 male New Zealand white rabbits weighing between 1800 g and 2000 g were randomly chosen from thousands of others at a commercial farm (East Asia Rabbit Corporation) located in Semenyih, West Malaysia where they had been kept in cages and fed commercial rabbit pellet. The rabbits were transported for less than 1 h to the abattoir at the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia where the slaughter was conducted. The animals were slaughtered according to Halal slaughter procedure as outlined in the Malaysian Standard MS1500: 2009 (Department of Standards Malaysia, 2009). The slaughter was performed by a licensed slaughter man by severing carotid artery, jugular vein, trachea and esophagus using a sharp knife.

2.3. Carcass sampling

After evisceration and carcass dressing, the left LL between the 6th and 8th lumbar vertebra was removed and divided into two, and snap frozen in liquid nitrogen before being stored at −80 °C. The first portion was assigned for subsequent determination of lipid and protein oxidation at d 0. The second portion was assigned for subsequent determination of TBARS at d 0. The right hind limbs and the fore arms were aseptically packed in stomacher bags and aerobically stored at 4 °C for microbial enumeration. The carcasses were then hung in the 4 °C chiller and the subsequent sampling was carried out at specific periods. The left LL muscle from the 9th to 12th lumbar vertebra was dissected into three portions at specific periods of 1, 3, 5 and 7 d post-mortem for subsequent analysis of TBARS. The right LL muscle that was divided into two parts from the 6th to 8th lumbar vertebra was used for the determination of ultimate pH. The portion from the 9th to 12th lumbar vertebra was further dissected at 3 specific periods, that is, 24 h and 7 and 14 d postmortem for determination of protein oxidation. Upon completion of each aging period, muscle chops of approximately 2.5 cm thickness were dissected from each specific muscle, labeled, vacuum packaged and stored at −80 °C until subsequent analyses.

2.4. Determination of pH

The pH of the meat was determined using a portable pH meter (Mettler Toledo, AG 8603, Switzerland) following the indirect method described by AMSA (2012). The samples were removed from −80 °C storage and manually pulverized in liquid nitrogen. Approximately 1.0 g of each crushed muscle sample was homogenized (Wiggen Hauser® D-500, Germany) for 30 s in 10 ml ice-cold deionized water in the presence of 5 mM sodium iodoacetate (Merck Schuchardt OHG, Germany) to prevent further glycolysis (specifically glyceraldehyde 3-phosphate dehydrogenase) or production of lactic acid (AMSA, 2012). The pH of the resultant homogenates was measured using the electrode attached to the pH meter.

2.5. Microbiological analysis

On each sampling day, 25 g of meat samples was aseptically weighed, transferred to a stomacher bag containing 225 ml of 0.1% of peptone water (Merck KGaA, Germany) and homogenized using a stomacher (Inter Science, France) for 120 s at room temperature. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10 diluent, and peptone water) of homogenates were spread on the surface of dry media. Tenfold dilutions were spread plated in duplicate. Aerobic plate counts were enumerated on plate count agar (Merck KGaA, Germany) following 2 d of incubation at 30 °C (Harrigan, 1998; Rodríguez-Calleja et al., 2010). *Pseudomonas aeruginosa* numbers were determined, after 2 d of incubation at 25 °C (Rodríguez-Calleja et al., 2010) on Fluka® Analytical 70887-500G Cetrimide Agar (SIGMA-ALDRICH, Spain). *Escherichia coli* were enumerated after 24 h of incubation on Tryptone Soy Agar (CM0131, Oxoid, England) at 36 °C. *Brochothrix thermosphacta* was enumerated on streptomycin sulfate cycloheximide thallous acetate agar (STAA, Oxoid), supplemented with STAA Selective Supplement SR0151 E (Oxoid, England) following 18 h of incubation at 26 °C.

2.6. Lipid oxidation measurement

Lipid oxidation was measured as 2-thiobarbituric acid reactive substances (TBARS) using the QuantiChrom™ TBARS Assay Kit (DTBA-100, BioAssay Systems, USA) following the method of Nakyinsige et al. (2014). Concisely, samples were manually pulverized in liquid nitrogen. About 200 mg of the pulverized samples was mixed with 2 ml ice-cold phosphate buffered saline (PBS) and rapidly homogenized with an Ultra-Turrax T5FU (IKA-Labortechnik Staufen, Germany) for 20 s on ice. Two hundred microliters of homogenates was mixed with 200 μl of ice-cold 10% trichloroacetic acid (TCA) and incubated on crushed ice for 5 min. This was followed by centrifugation (Eppendorf Centrifuge, Mikro 22R Hettich, Germany) at 21,900 *g*, 4 °C for 5 min. Standards were prepared by mixing 15 μl of the 1.5 mM malondialdehyde (MDA) with 735 μl deionized water to obtain a final concentration of 30 μM MDA. Subsequently, 300, 180, 90 and 0 μl of 30 μM MDA were diluted with 0, 120, 210 and 300 μl of deionized water to generate the final 30, 18, 9 and 0 μM MDA as standards 1, 2, 3 and 4, respectively. Exactly 200 μl of thiobarbituric acid reagent was added to 200 μl of samples and standards and the mixture was incubated in a dry heating block at 100 °C for 60 min. Following equilibration to room temperature, 100 μl of standards and samples was loaded in duplicates into wells of a clear flat- bottom 96-well plate (Greiner Bio-One, Germany). Finally, optical density (OD) was determined at 535 nm (OD_{535}) using an auto UV Xenon flash lamp microplate reader (infinite M200, Tecan, Austria). After subtracting the OD of blank (standard 4) from all standard and sample values, a standard curve was obtained by plotting the ΔOD_{535} against standard concentrations. TBARS (μM MDA equivalent) concentration of