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Changes in the quality of superchilled rabbit meat stored at different temperatures

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ABSTRACT

This work studied the effects of a superchilling process at two different temperatures on the shelf life and selected quality parameters of rabbit meat. As the storage time increased, the rates at which the total aerobic count, total volatile basic nitrogen, thiobarbituric acid-reactive substances and pH value increased were significantly lower in superchilled rabbit meat stored at −4 °C compared to those in rabbit meat stored at −2.5 °C and 4 °C. SDS-PAGE analysis indicated that the decrease in storage temperature could significantly reduce the degree of protein degradation. The lightness, redness, shear force, the integrity of muscle microstructure and water holding capacity decreased with increasing storage time. Compared with the samples frozen at −18 °C, superchilled rabbit meat shows a marked reduction in microstructure deterioration. These results suggest that shelf life of good-quality rabbit meat was 20 d under superchilling at −2.5 °C and at least 36 d under superchilling at −4 °C, compared with less than 6 d under traditional chilled storage.

1. Introduction

Rabbit meat is an important product from an economical and nutritious perspective. China is one of the largest producers and consumers throughout the world. Over the past decade, China's production of rabbit meat has doubled, reaching 723,975 tons in 2013 and accounting for 41.6% of global production (FAOSTAT, 2013). Rabbit meat is one of the healthiest meats because of its nutritive and dietetic properties, such as low fat content, low allergenicity and cholesterol, high digestibility and unsaturated fatty acid content (Dalle Zotte & Szendro, 2011; Vergara, Berruga, & Linares, 2005).

The superchilling process was described as early as 1920 by Le Danois, and the terms "superchilling", "partial freezing" and "deep chilling" are used to describe a process by which food products are stored between their initial freezing point (−0.5 °C to −2.8 °C for most food) and 1–2 °C below this temperature in most reports (Chang, Chang, Shiau, & Pan, 1998; Duun & Rustad, 2007; Magnussen, Haugland, Hemmingsen, Johansen, & Nordtvedt, 2008). Compared with traditional chilling technology, superchilling can retain better food quality and prolong the shelf life of most stored food by at least 1.5–4 times (Kaale, Eikevik, Rustad, & Kolsaker, 2011); it can also reduce the use of freezing/thawing and thereby a lower energy cost was involved in superchilling in terms of transportation and retailing compared with freezing technology (Zhou, Xu, & Liu, 2010).

Previous studies have investigated the effects of superchilling on the shelf life and quality parameters of aquatic products only at a special temperature (Gallart-Jornet, Rustad, Barat, Fito, & Escriche, 2007; Kaale, Eikevik, Rustad, & Nordtvedt, 2014; Beaufort, Cardinal, Le-Bail, & Midelet-Bourdin, 2009; Fernandez, Aspe, & Roeckel, 2009; Olafsdottir, Lauzon, Martinsdottir, Oehlenschlager, & Kristbergsson, 2006). Sivertsvik, Rosnes, and Kleiberg (2003) reported that a 21-day sensory shelf life was for superchilled (−2 °C) salmon in air, whereas chilled (4 °C) filets were spoiled after 7 d. A combination of superchilling with modified atmosphere packaging (MAP) or vacuum packaging can greatly extend the shelf life (Duun & Rustad, 2007; Wang, Sveinsdottir, Magnusson, & Martinsdottir, 2008). The formation and growth of intra- and extracellular ice crystals during superchilling storage are significant. These crystals accelerate the extent of myofiber detachment and breakage (Bahuaud et al., 2008; Kaale, Eikevik, Bardal, Kjorsvik, & Nordtvedt, 2013). However, there are few published studies describing the effect of different superchilling temperatures on the shelf life and microstructure of livestock products, and to the best of our knowledge, no literature concerning rabbit meat has yet been published. The purpose of this study was to investigate the effects of different superchilling temperatures (−4 °C and −2.5 °C) on the shelf life, sensory and nutritional quality parameters of rabbit meat, including total aerobic count, total volatile basic nitrogen, color, tenderness and protein structure. At the same time, we also observed the effect of the growth of ice crystals on the microstructure of rabbit muscles at different freezing temperatures.

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2. Materials and methods

2.1. Materials

Forty male Ira rabbits (2–2.3 kg/live rabbit) from the experimental farm of the Southwest University (Chongqing, China) were slaughtered by standard commercial procedures, and the head, viscera and skin were removed. The carcasses were immediately transferred to chilled insulated EPS boxes (approximately 6 °C) and then transported to laboratory within 1 h after killing. Unstained protein ladder was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Coomassie Brilliant Blue R250 and glycine used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All chemicals and reagents were of analytical grade.

2.2. Sample preparation

The carcasses were chilled at 4 °C for approximately 24 h until rigor mortis dissipated, and the hind legs were then dissected. Every leg was weighed, placed in a polypropylene tray and wrapped with a low-oxygen-permeable polyvinyl dichloride film (thickness of 0.25 mm and oxygen permeability of 60 cm^3/m^2/24 h/atm; Shiny-day Group Co., Ltd., Hainan, China). Samples were subsequently cooled in a refrigeration cabinet at a temperature of −30 °C until the core temperature was approximately −2 °C (about 80 min) and then immediately transferred to a cold storage room at −2.5 ± 0.3 °C or −4 ± 0.4 °C for temperature equalization and storage for up to 36 d (freezing point of Ira rabbit hind legs is between −1.8 °C and −2.1 °C). The day of processing was denoted day 0. Air temperature was recorded every 1 min during superchilled storage by loggers with an internal sensor (L91-1+, Hangzhou Loggertech Co., Ltd., Zhejiang, China). References were stored at 4 ± 1 °C for up to 10 d or frozen at −18 °C for 36 d. Prior to analysis, samples stored at sub-zero temperatures were transferred to storage at 4 °C and thawed for 12 h. Superchilled samples were analyzed on days 4, 8, 12, 16, 20, 24, 28, 32 and 36, whereas chilled references stored at 4 °C were analyzed on days 0, 2, 4, 6, 8, and 10, and frozen references stored at −18 °C were analyzed on day 36.

2.3. Microbiological analysis

The determination of the total aerobic count (TAC) was performed as described by Liu, Kong, Han, Chen, and He (2014). Ten grams of minced rabbit meat from each treatment was weighed aseptically and homogenized with 90 mL of 0.85% sterile physiological saline for 1 min. Serial tenfold dilutions were performed by adding 1 mL homogenates to 9 mL of 0.85% sterile physiological saline. The pour plates of the appropriate dilutions were incubated at 36 °C ± 1 °C for 48 h. All counts were expressed as log CFU/g.

2.4. pH value

A sample of 3 g minced rabbit was mixed with 30 mL KCl (pH 7.0 0.1 mol/L) and homogenized by a homogenizer (XHF-D, Ningbo Scientz Biotechnology Inc., Zhejiang, China) for 1 min at 6000 rpm. The pH of the mixtures was measured by a digital pH meter (PHS-3+, Century Fangzhou Science & Technology Co., Ltd., Chengdu, China).

2.5. Total volatile basic nitrogen

Total volatile basic nitrogen (TVB-N) was measured by semi-microsteam distillation, as described by Zhang, Li, Lu, Shen, and Luo (2011) with slight modifications. A sample of 10 g minced rabbit was dispersed in 100 mL distilled water and stirred for 30 min, and the mixture was then filtered. The TVB-N value was determined according to the consumption of hydrochloric acid and calculated using the following equation: $\text{TVB-N}(\text{mg}/100\,\text{g}) = (Y_1 - Y_2) \times C \times 2800$, where Y_1 is the titration volume of hydrochloric acid in the sample, Y_2 is the titration volume in the blank, and C is the concentration of hydrochloric acid (0.01 mol/L).

2.6. Thiobarbituric acid-reactive substances

Lipid oxidation was evaluated by thiobarbituric acid-reactive substances (TBARS) according to the procedure developed by Lo Fiego et al. (2004) with some modifications. A sample of 10 g minced rabbit was mixed with 20 mL of 20% trichloroacetic acid (TCA) and homogenized (XHF-D, Ningbo Scientz Biotechnology Inc., Zhejiang, China) at 6000 rpm for 1 min. After centrifugation (5500 rpm for 15 min at 4 °C), the supernatant was filtered through filter paper. Five milliliters of filtrate was combined with 5 mL of 0.02 mol/L 2-thiobarbituric acid solutions (TBA) solutions and heated in a boiling water bath for 20 min together with a blank containing 5 mL of 20% TCA and 5 mL TBA reagent. After the resulting solution was cooled under running water for 10 min, the solution's absorbance was measured at 532 nm with a spectrophotometer (Model 722, Jinghua Science & Technology Co., Ltd., Shanghai, China). The TBARS value was expressed as mg of malondialdehyde/kg of rabbit sample and calculated using the following equation: $\text{TBARS}\ (\text{mg/kg}) = (A_{532} + 0.002) \times 2.587$, where A_{532} is the absorbance value of the assay solution.

2.7. Protein electrophoresis

Soluble protein was extracted according to the method described by Duun and Rustad (2008), and the amount of soluble protein was determined by Biuret colorimetry as described by Zheng et al. (2013). The degradation of the salt soluble protein was analyzed by SDS-PAGE according to the method described by Laemmli (1970) with some modifications. All equipment for electrophoresis was purchased from Bio-Rad Laboratories (Richmond, CA, USA). One milliliter of 1 mg/mL protein sample was mixed with 1 mL sample buffer (0.1 g SDS, 0.1 mL β-mercaptoethanol, 0.002 g bromophenol blue, 2 g glycerol and 2 mL Tris–HCl of 0.05 mol/L pH 8.0 were mixed and deionized water was added to a total volume of 10 mL). The mixture was heated in boiling water for 3 min and subsequently centrifuged at 1800 *g* for 10 min. The concentrations of the running gel and stacking gel were 12% and 4%, respectively, and 20 μL of supernatant was loaded into each well on the gel, but only 5 μL unstained protein ladder was loaded into one well. The stacking gel and running gel were run on a Bio-Rad Mini-Protein II system at constant currents of 15 mA and 30 mA, respectively, and the total run time was approximately 2.5 h. The gels were stained for protein with Coomassie Brilliant Blue R250 and scanned using a Gel Imaging System (G:BOX, Genesys, California, USA).

2.8. Drip loss

The drip loss of the hind legs was estimated as described by Lauzon, Magnusson, Sveinsdottir, Gudjonsdottir, and Martinsdottir (2009) and calculated by the following equation: drip loss (%) = (initial weight raw material − weight after thawing) / (initial weight raw material) × 100.

2.9. Cooking loss

Samples measuring 5 × 5 × 2 cm were cut from the hind legs, wrapped in a retort pouch, and subsequently immersed completely in a constant-temperature water bath at 70 °C for 30 min (Honikel, 1998). After cooking, samples were removed and cooled to room temperature under tap water for 20 min. The cooking loss was estimated as described by Kim et al. (2013) and calculated by the following equation: cooking loss (%) = (weight of uncooked sample − weight of cooked sample) / (weight of uncooked sample) × 100.