Meat Science 121 (2016) 253–260

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Changes in the microbiota of lamb packaged in a vacuum and in modified atmospheres during chilled storage analysed by high-throughput sequencing

Taojun Wang [a], Liang Zhao [b], Yanan Sun [a], Fazheng Ren [a,c], Shanbin Chen [a], Hao Zhang [b], Huiyuan Guo [a,b,*]

[a] Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] Beijing Higher Institution Engineering Research Center of Animal Product, Beijing 100083, China
[c] Beijing Laboratory for Food Quality and Safety, Beijing 100083, China

ARTICLE INFO

Article history:
Received 30 December 2015
Received in revised form 15 June 2016
Accepted 17 June 2016
Available online 18 June 2016

Keywords:
CO_2
High-throughput sequencing
Lamb
Microbiota
Modified atmosphere packaging

ABSTRACT

Changes in the microbiota of lamb were investigated under vacuum packaging (VP) and under 20% CO_2/80% N_2 (LC), 60% CO_2/40% N_2 (MC), and 100% CO_2 (HC) modified atmosphere packaging (MAP) during chilled storage. Viable counts were monitored, and the total microbial communities were assessed by high-throughput sequencing. The starting community had the highest microbial diversity, after which *Lactococcus* and *Carnobacterium* spp. outcompeted during the 28-day storage. The relative abundances of *Brochothrix* spp. in the LC atmosphere were much higher than those of the other groups on days 7 and 28. The bacterial inhibiting effect of the MAP environments on microbial growth was positively correlated with the CO_2 concentration. The HC atmosphere inhibited microbial growth and delayed changes in the microbial community composition, extending the lamb's shelf life by approximately 7 days compared with the VP atmosphere. Lamb packaged in the VP atmosphere had a more desirable colour but a higher weight loss than lamb packaged in the MAP atmospheres.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

China was the world's largest producer of lamb in 2011, and in 2013, lamb meat production reached 4.08 million tonnes. Today, lamb production in China is continuing to increase (L. Sun, Zhao, Zhan, & Jiang, 2014; Y. Zhou, Al, & Zhang, 2015). However, lamb is susceptible to microbial spoilage because of its nutrient-rich and high water contents (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015). Deleterious effects, such as discolouration, unpleasant odours, and slime production, can be caused by microbial growth, especially by bacteria with high spoilage potential (Singh, Wani, Saengerlaub, & Langowski, 2011; X. D. Sun & Holley, 2012). Therefore, modified atmosphere packaging (MAP) that can inhibit microbes is a good strategy for prolonging meat shelf life and maintaining meat quality (Arvanitoyannis & Stratakos, 2012; G. H. Zhou, Xu, & Liu, 2010).

It has been reported that the main gases used in MAP environments to preserve food products are N_2, O_2, and CO_2 and that the extension of the shelf life of fresh meat mainly depends on the bacterial inhibiting effect of CO_2 (Arvanitoyannis & Stratakos, 2012; Jakobsen & Bertelsen, 2002; Oses et al., 2013). Hence, it is important to have a clear understanding of the effects of CO_2 on the microbial community of lamb meat. Previous research on lamb meat product packaged in 30% CO_2/70% N_2 and 70% CO_2/30% N_2 MAP environments has indicated that the 70% CO_2/30% N_2 environment more effectively inhibits the total viable counts (TVCs), *Pseudomonas* spp., and *Brochothrix thermosphacta* than the 30% CO_2/70% N_2 environment. Furthermore, the shelf life of this lamb product was extended by approximately 4–5 days compared to lamb product packaged in aerobic conditions (Soldatou, Nerantzaki, Kontominas, & Savvaidis, 2009). In another study, lamb steaks packaged in 100% CO_2, 90% CO_2/10% N_2 and 80% CO_2/20% N_2 MAP environments were studied, and the results indicated that the lamb packaged in 100% CO_2 had the lowest viable counts of *Pseudomonas* spp. and coliform bacteria (Kennedy, Buckley, & Kerry, 2005). However, the previous reports on the bacterial inhibiting effect of CO_2 mainly used culture-dependent methods to assess the microbial loads during storage. Such methods are labour-intensive and time-consuming, which limits their ability to assess the total microbial community of lamb (Jasson, Jacxsens, Luning, Rajkovic, & Uyttendaele, 2010).

Molecular methods are rapid and reliable ways to analyse microbial communities. Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) has been used widely to investigate changes in microbial communities during the storage of meat and meat products (Audenaert et al., 2010; Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Fontana, Cocconcelli, & Vignolo, 2006). In recent years, as noted by

* Corresponding author at: College of Food Science & Nutritional Engineering, China Agricultural University, Beijing 100083, China.
E-mail address: guohuiyuan@cau.edu.cn (H. Guo).

http://dx.doi.org/10.1016/j.meatsci.2016.06.021
0309-1740/© 2016 Elsevier Ltd. All rights reserved.

Polka, Rebecchi, Pisacane, Morelli, and Puglisi (2015), advancements in high-throughput sequencing are revolutionizing our ability to assess and understand microbial communities. High-throughput sequencing is more powerful than PCR-DGGE, and not only can it obtain complete coverage of microbial communities, but it can also provide a quantitative estimate of the abundance of single taxa in each sample based on the generation of multimillion-sequence reads (Doulgeraki, Ercolini, Villani, & Nychas, 2012; Nam, Yi, & Lim, 2012; Polka et al., 2015; Zhao et al., 2015). However, to the best of our knowledge, the microbial communities of lamb under MAP environments with different CO_2 concentrations have not been investigated by high-throughput sequencing.

The aim of this study was to investigate the total microbial community compositions of lamb packaged in vacuum and MAP environments with different CO_2 concentrations using high-throughput sequencing and to assess the viable counts using plate counting. Meanwhile, physicochemical changes in the meat were measured to assess the effects of the MAP with different CO_2 concentrations and vacuum environments on meat quality during storage.

2. Materials and methods

2.1. Sample collection

Lamb hind legs were collected at a local commercial abattoir (ZhuoChen, Beijing, China) 24 h after slaughter. Sixty-four samples were divided into four groups (16 samples each). The samples of one group were individually packaged in vacuum using a vacuum-packaging machine (DZ-600/2S, Xiaokang, Shandong, China). The samples of the other three groups were individually placed in rigid plastic trays (TQBC-1175, Sealed Air Corporation, Saddle Brook, USA) with an O_2 transmission rate of 10 cm^{-3} m^{-2} day^{-1} atm^{-1} at 23 °C and were subsequently packaged in 20% CO_2/80% N_2 (low CO_2 (LC) concentration), 60% CO_2/40% N_2 (middle CO_2 (MC) concentration), and 100% CO_2 (high CO_2 (HC) concentration) environments respectively using a modified atmosphere packaging machine (HT-300, Hengxian, Beijing, China). All samples were stored at 4 °C for 28 days. An analysis (four samples) on day 0 (Air.0d) was performed prior to packaging, and the data were the same for all of the groups. Samples packaged in the vacuum and in the three modified atmospheres (four samples for each group) were analysed at 7, 14, 21, and 28 days during chilled storage.

2.2. Gas measurement

In practice, it is almost impossible to establish MAP environments that are completely free of O_2. Hence, prior to further analyses, the concentrations of CO_2 and residual O_2 were measured by a headspace gas analyser (Checkmate 3 O_2 (Zr) CO_2-100%; DK-4100, Dansensor, Ringsted, Denmark) at each sampling point, and the remaining gas was considered to be N_2. The headspace gas was drawn off by a syringe needle attached to the machine through a septum that was glued onto the product surface.

2.3. Meat quality

pH values were measured with a meat pH meter (HI 99163, Hanna Instruments Ltd., Padova, Italy), and the pH probe was calibrated prior to each sampling session. Colour values were measured by a spectrophotometer (CM-700d, Konica Minolta, Inc., Tokyo, Japan) with illuminant D65, a 0° viewing angle and an 8-mm viewing area. The spectrophotometer was calibrated with white standard plates before analysis (CM-A177). When opening the packages, the colour coordinates of L* (lightness), a* (redness), and b* (yellowness) were recorded at each sampling point. Weight loss was measured as described previously (Ercolini et al., 2006). Lamb meat was weighed prior to packaging and at each storage time. Then, the difference in the weight (g) was divided by the initial weight of the lamb meat to calculate the percentage of weight loss. For the indicators of meat quality, four repetitions were performed on each sample at each sampling point when opening the package.

2.4. Enumeration of microorganisms

Twenty-five grams of lamb meat from each sample was diluted aseptically in 225 mL of sterile saline (8.5 g/L NaCl) and homogenized for 1 min using a stomacher. Serial decimal dilutions were prepared, and suitable dilutions were incubated in duplicate on selective media. TVCs were determined on plate count agar (PCA, Aoboxing, Beijing, China) after incubation at 30 °C for 48 h. Lactic acid bacteria (LAB) were plated on De Man, Rogosa, and Sharpe agar (MRS, Beijing, Aoboxing) and incubated anaerobically at 30 °C for 48 h. *Pseudomonas* spp. were incubated on *Pseudomonas* selective agar (PSA, Haibo, Qingdao, China) supplemented with CFC (Cetrimide, Fucidin, Cephaloridine, Haibo) at 30 °C for 48 h. *Enterobacteriaceae* were grown on violet red bile glucose agar (VRBGA, Haibo) and incubated at 37 °C for 24 h. After incubation, the numbers of colony-forming units (CFU) were counted.

2.5. DNA extraction and high-throughput sequencing

Ten grams of meat from each sample was placed into a sterile tube containing 20 mL of phosphate buffered solution (PBS) and agitated for 5 min. The tube was centrifuged for 5 min at 200 $\times g$ to separate large debris, and the supernatant was transferred to a new sterile tube for further centrifugation at 12,000 $\times g$ for 10 min to harvest the bacteria. Bacterial genomic DNA was extracted using the phenol-chloroform method as described previously (Via & Falkinham, 1995). DNA samples from four samples for each group at each sampling point were mixed together for high-throughput sequencing.

The specific primers 341F (5′–CCTAYGGGRBGCASCAG–3′) and 806R (5′–GGACTACNNGGGTATCTAAT–3′) with a barcode were used to amplify the V3-V4 region of the 16S rRNA gene to identify the microbial species (Polka et al., 2015). PCRs were performed in 30-μL volumes consisting of 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 0.2 μM primers, and 10 ng of template DNA. The amplification conditions were as follows: initial denaturation at 98 °C for 1 min; followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 60 s; followed by a final extension at 72 °C for 5 min. The PCR products were detected by 2% agarose gel electrophoresis and purified with the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, the purified products were used to generate sequencing libraries with the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs) according to the manufacturer's recommendations. The library was sequenced on the Illumina HiSeq platform, and 250-bp paired-end reads were generated (Novogene, Beijing, China).

2.6. Data analysis

The raw sequencing data were merged using the Fast Length Adjustment of SHort reads (FLASH) function to obtain raw tags (Magoc & Salzberg, 2011). Then, the raw tags were screened by the Quantitative Insights Into Microbial Ecology (QIIME) function (Caporaso et al., 2010) to obtain clean tags. UCHIME was used to remove chimeras to obtain effective tags (Edgar, Haas, Clemente, Quince, & Knight, 2011). The effective tags, at an identity threshold of 97%, were assigned to the same operational taxonomic units (OTUs) with UPARSE software (Edgar, 2013). Representative sequences for each OTU were submitted to the Ribosomal Database Project (RDP) classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier/) to obtain the taxonomy assignment and the relative abundance of each OTU using the Greengenes database (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi) (DeSantis et

Download English Version:

https://daneshyari.com/en/article/5791086

Download Persian Version:

https://daneshyari.com/article/5791086

Daneshyari.com