Effects of carbon dioxide in high-oxygen modified atmosphere packaging on Pseudomonas growth and beef spoilage

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Introduction: Beef is one of the most perishable foods caused by the growth of specific spoilage organisms (SSOs), such as Pseudomonas and Brochothrix[1]. High oxygen modified atmosphere packaging (HiOx-MAP) with 50% O2/40% CO2/10% N2 can extend the shelf-life of chilled beef to 20 days, which is attributed to the antibacterial effect of CO2[2]. Numerous studies have indicated that Pseudomonas are more sensitive to CO2 than other SSOs. However, there are some different conclusions about the inhibitory effects of CO2 on Pseudomonas species. This study aimed to evaluate the bacteriostatic effects of CO2 on Pseudomonas to clarify the response and promote the wider application of MAP technology.

Materials and methods: The M. longissimus lumborum were collected from four Simmental cattle in an abattoir. Both left and right loins were cut into 2.54-cm steaks and two steaks from each carcass were randomly assigned to TMAP (50% O2/40% CO2/10% N2) or CMAP (50% O2/50% N2) for each storage times (5, 10, 15, and 20 d), and two steaks from each carcass were analyzed at day 0 (n = 8). All packages stored in a chiller at 2 ℃. For each time point, total viable counts (TVC), Pseudomonas counts and total volatile basic nitrogen (TVB-N) were measured. The diversity assessment of Pseudomonas was analyzed with 16S rRNA gene sequencing at day 20. Afterwards, the spoilage potential of P. fragi was calculated by yield factor pYTVB-N/CFU = -log (TVB-Nfinal - TVB-Ninitial/ CFUfinal - CFUinitial)[3]. The MIXED procedure of SAS Version 9.0 was used with packaging type, storage time and their interaction as fixed factors and carcass as a random factor. Least squares means were separated using the PDIFF option and were considered significant at $P < 0.05$.

Results: There was a significant packaging × storage time interaction for TVC, Pseudomonas and TVB-N values. The initial TVC was 4.94 log CFU/g, and in the CMAP steaks increased from day 5 ($P < 0.05$) and exceeded 7 log CFU/g at day 20. The growth of Pseudomonas were similar with TVC. In a related manner, the TVB-N values in the CMAP steaks increased as storage time extended and exceeded the spoilage threshold (≤15 mg/100 g) at the end of storage. By comparison the values of TVC, Pseudomonas and TVB-N in TMAP steaks only increased by 0.25 log CFU/g, 0.74 log CFU/g and 1.2 mg/100g, respectively.

P. fragi was the dominant species under CMAP (28/40), and others were determined as P. weihenstephanensis (5/40), P. psychrophile (1/40), P. versuta (6/40). However, in the TMAP steaks, P. fragi was significantly inhibited by CO2 (4/40) and P. versuta was present in a higher abundance (30/40), which has been pointed without tributyrin lipolysis[4]. Other isolates were determined as P. fluorescens (3/40), P. lundensis (1/40), P. weihenstephanensis (1/40) and P. taetrolens (1/40) in TMAP steaks. P. fragi is regarded as the most dominant species on chilled meat with a high spoilage potential, while the spoilage role of P. versuta in beef is poorly understood. The value of pYTVB-N/ CFU of P. fragi were various among the isolates in our case, and the smaller the pYTVB-N/CFU, the stronger the spoilage potential. Therefore, the isolate T1 in TMAP had the strongest spoilage potential. Comparation of spoilage role between P. fragi and P. versuta in beef during storage will be worthy of investigation.

Conclusions: In this study, the presence of CO2 (40% CO2) in HiOx-MAP significantly reduced TVC and Pseudomonas counts, and decreased the production of TVB-N, as well as extended the shelf-life of steaks. P. fragi, the dominant bacteria in CMAP steaks at day 20, were more sensitive to the CO2 inhibition than other species, meanwhile P. versuta showed a higher abundance in TMAP, which may be beneficial for retarding the beef spoilage process.

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