Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.100522

Effects of different storage temperatures on microbial spoilage and bacterial community structure of fresh beef by high-throughput sequencing technology

Xinhui WANG[1*#], Tingyang ZHANG[1#], Yidan YANG[1#], Lijia LIU[1], Tian TIAN[2*], Dandan ZHU[1], Maolan MA[1], Simin XIE[1]

Abstract

Comparsion of the effects of the chilling (4 ºC), superchilling (-2 ºC) and frozen (-18 ºC) storage on microbial spoilage and bacterial community structure of fresh beef was evaluated by high-throughput sequencing technology in this study. The results indicated that storage temperature can significantly affect the degree of microbial spoilage and bacterial community structure of fresh beef during storage. At the species level, the primary dominant bacterial species in beef samples was *Pseudomonas fragi* with relative abundance of 37.78% followed by *Myroides phaeus* and *Brochothrix thermosphacta* with 10.95% and 4.64%, respectively, at the beginning of storage. Both superchilling and frozen storage can effectively inhibit the growth of *P. fragi*, while the chilling storage failed to demonstrate strong inhibition for growth of *P. fragi*. Similar result was found for *Myroides phaeus*. The chilling, superchilling and frozen storage can not effectively inhibit the growth of *Brochothrix thermosphacta* and *Acinetobacter johnsonii*. The difference of microbial spoilage in beef stored in three kinds of storage may be related to the difference of the bacterial community structure and the formation of dominant spoilage bacteria. These results revealed that superchilling and frozen storage can effectively inhibit the formation of dominant spoilage bacteria, resulting in a good freshness preservation.

Keywords: bacterial community structure; freshness preservation; high-throughput sequencing; storage temperature.

Practical Application: In this study, comparsion of the effects of the chilling (4 ºC), superchilling (-2 ºC) and frozen (-18 ºC) storage on microbial spoilage and bacterial community structure of fresh beef was evaluated by high-throughput sequencing technology. These results revealed that sperchilling and frozen storage can effectively inhibit the formation of dominant spoilage bacteria (*Pseudomonas fragi*), resulting in a good freshness preservation.

1 Introduction

Fresh meat is very conducive to the microbial growth because of its high water content, optimal pH and abundant nutrients (Liang et al., 2021), which will lead to the spoilage of fresh meat. The spoilage degree is closely related to the species and amount of bacteria under specific storage conditions (Kaur et al., 2021; Li et al., 2019; Mansur et al., 2019). Thus, the bacterial communities play an important role in spoilage of meat. For fresh meat, the storage temperature is the most important factor affecting the microbial growth and bacterial community structure, which can determine the shelf life and quality of meat. Currently, the chilling storage (0-4 ºC), superchilling storage (-1--2 ºC) and frozen storage (-18--40 ºC) are the most commonly commercial storage (Pan et al., 2019). To date, there have been many studies focused on the quality of fresh meat under different storage, but microbial community structure under different storages has rarely been reported.

Nowadays, the high-throughput sequencing (HTS) technology appeared leading to characterize more precisely microbial diversity in foods compared with the culture dependent methods. It has been reported that HTS technology is a powerful tool for exploring natural diversity because it can generate thousands of sequences within a short time to cover the complex microbial communities as well as low abundance microorganisms (Wang et al., 2018b). Thus, this technology has been used to explore the bacterial composition in foods.

Thus, in this study, three kinds of storage method, namely chilling storage (4 ºC), superchilling storage (-2 ºC) and frozen storage (-18 ºC), were performed to store the fresh beef. Comparsion of the effects of the chilling, superchilling and frozen storage on microbial spoilage and bacterial community structure of fresh beef was evaluated by high-throughput sequencing technology to determine the poilage related bacterial communities and dominant spoilage bacteria. Additionally, the potential mechanism of spoilage bacteria was investigated by functional analysis.

2 Materials and methods

2.1 Sample processing

Fresh beef *longissimus lumborum* muscle was supplied from a local abattoirs located in Chengdu, Sichuan Province, China, within 12 h after slaughter. Then the fresh beef was divided

Received 25 Aug., 2022
Accepted 21 Oct., 2022
[1] *Meat-Processing Application Key Laboratory of Sichuan Province, School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan, China*
[2] *Chengdu Agricultural College, Chengdu, Sichuan, China*
*Corresponding author: wangxinhui19820319@163.com; 250854469@qq.com
#Equal contribution

into samples (10 cm × 10 cm × 1.5 cm) with about weight of 100 g for experiments. Subsequently, all samples were equally divided into three groups and each group contains 30 samples for experiments. Samples stored in chilling (4 °C), superchilling (-2 °C) and frozen (-18 °C) conditions were labeled as Group C, Group S and , Group F, respectively.

2.2 Physicochemical parameters determination

The pH values of beef samples were measured according to the method described by Wang et al. (2015b) using a pH meter (Testo 205, Testo International Trade Co., Ltd., Shenzhen, China) with automatic temperature compensation (NTC) electrode. The total volatile basic nitrogen (TVB-N) concentration was measured in accordance to Chinese standard protocols GB/T 5009.228-2016 (National Health and Family Planning Commission of China, 2017) and was expressed as mg/100 g sample. Drip loss was measured according to the method described by Wang et al (2022a). The color of beef samples was measured according to the method described by Wang et al. (2015a) using an auto color chromameter (CS-22, Hangzhou CHNSpec Technology Co. Ltd, Hangzhou, China).

2.3 Total Viable Counts (TVC) determination

Total viable counts (TVC) was measured in accordance to the method described by Wang et al. (2021). Briefly, 5 g beef sample was weighed accurately by using sterile scissors and added into a sampling bag containing 95 mL of sterile saline and was homogenized for 1 min.Then the mixture was serially diluted with sterile saline and 0.1 mL of diluent was plated onto agar plates (CM101, Beijing Luqiao Technology, Beijing, China). The plates were incubated at 36 ± 1 °C for 48 h for colony forming units (CFU) counting. The results were expressed in lg CFU/g.

2.4 Bacterial community structure analysis

DNA extraction, PCR amplification and sequencing

Total microbial DNA in the samples was extracted according to the method described by Wang et al. (2018b) using the E.Z.N.ATM Mag-Bind Soil DNA Kit (OMEGA, USA). After extraction and purification, the DNA concentration was determined according to the guarantee values of OD_{260}/OD_{280} and OD_{260}/OD_{230} above 1.8 and 2.0, respectively, and 1.5% agarose gel was used to check the DNA quality (Wang et al., 2018b).

The V4 region of bacterial 16S rRNA gene was amplified with the primer pairs 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') and the DNA extraction was used as the template (Wang et al., 2018a). The PCR amplification consisted of a two-step PCR technology and the systems and conditions of PCR amplification were undertaken according to the method described by Wang et al. (2018a). After amplification, the PCR products were purified and quantified, and were sequenced on the llumina MiSeq platform (Beijing Novogene Technology Co. Ltd, Beijing, China).

Processing of sequencing data

The raw 16S rRNA gene sequencing reads were merged and screened according to the method described by Cheng et al. (2018). Then the high quality sequences were clustered and regarded as operational clustering method (OTUs) at an identity threshold of 97%.

2.5 Statistical analysis

Each test was performed in triplicate. Data were displayed as mean values accompanied with the standard deviation. Duncan's multiple range test was performed to verify significant differences ($p < 0.05$) among samples.

3 Results and discussion

3.1 Effects of different storage temperatures on freshness of beef during storage

The pH value, color, TVB-N and drip loss were important indices to reflect the freshness of meat (Wang et al., 2022b; Rumape et al., 2022; Tian et al., 2022b). The four indices of beef during chilling, superchilling and frozen storage on the 7th day during storage are shown in Table 1.

The pH value of sample stored in chilling condition was the highest and was close to 6.0. In contrast, the pH values of samples stored in superchilling and frozen condition were 5.76 and 5.56, respectively. Any deviations from the normal in terms of pH will affect color intensity and water-holding capacity of fresh meat (Ding et al., 2020; Wen et al., 2022). Once the pH is over 6, the fresh beef appeared a undesirable quality acceptability. These results revealed that the superchilling and frozen storage effectively inhibited the increase of pH value compared to chilled storage, which was conducive to freshness maintenance.

The degree of *a*∗ of chilled beef is associated with consumer-defined beef color acceptability (Holman et al., 2017). Once the a* is lower than 14.5, the beef color will be considered unacceptable (Holman et al., 2017; Tian et al., 2022a). As shown in Table 1, the a* of beef stored in chilling condition was only 8.14, which reached the rejection level on the 7th day. In contrast, the a* of beef stored in superchilling and frozen condition was 35.15 and 34.46 on the 7th day, respectively, suggesting a desirable color acceptability. These results revealed that superchilling and frozen storage effectively maintained the beef color.

As shown in Table 1, the TVB-N concentration of beef stored in chilling condition reached 36.52 mg/100 g on the 7th day. In contrast, the TVB-N concentration of beef stored in superchilling and frozen condition was 13.56 mg/100 g and

Table 1. The pH value, color (a*), total volatile basic nitrogen (TVB-N) and drip loss of freh beef during different storage on the 7th day.

	Chilling storage	Superchiling storage	Frozen storage
pH	5.98 ± 0.056	5.76 ± 0.054	5.56 ± 0.025
Color (a*)	8.41 ± 1.20	35.15 ± 1.58	34.46 ± 0.69
TVB-N (mg/100 g)	36.52 ± 0.26	13.56 ± 0.11	13.36 ± 0.33
Drip loss (%)	3.15 ± 0.057	2.47 ± 0.098	3.24 ± 0.082

13.36 mg/100 g on the 7th day, respectively. 15 mg/100 g of TVB-N concentration has been National Food Safety Standard of China (GB 2707-2016) set as the upper limit for fresh level of meat (Pomponio et al., 2018; Holman et al., 2016). These results indicated that the shelf life of beef stored in superchilling and frozen condition was longer than 7 days and obviously displayed a longer shelf life than that of chilled storage.

The drip loss of beef stored in superchilling condition presented a lowest value among three kinds of samples on the 7th day as shown in Table 1, suggesting a good water-holding capacity. The water-holding capacity reduction was mainly attributed to the microstructure of muscle fibers damage (Li et al., 2020). In contrast to chilling and frozen storage, the superchilling storage can not only inhibit the activity of endogenous enzymes and exogenous microorganisms, but also reduce ice crystal formation during storage. Hence, the superchilling storage is instrumental in maintaining better water holding capacity.

Based on the results from the pH value, color, TVB-N and drip loss, the superchilling storage was conducive to prolonging the shelf life of beef with good quality compared to chilling and frozen storage, and is a good way to preserve freshness of beef within 7 days.

3.2 Effects of different storage temperatures on microbial quality of beef during storage

These results of bacterial enumeration of all samples stored in three different temperature conditions are shown in Table 2. The initial bacterial TVC value in all beef was approximately 3.0 lg CFU/g, suggesting a good hygienic quality of all tested samples. As storage time extended, the TVC value of samples stored in schilling condition increased sharply and reached to 7.78 lg CFU/g on the 7th day. In contrast to chilling storage, the TVC value of samples stored in superchilling and frozen condition increased slowly and reached to 4.82 lg CFU/g and 3.46 lg CFU/g on the 7th day, respectively. The 7 $\log_{10}$ CFU/g has been defined as threshold of microorganism counts for good quality fresh meat by the International Commission on Microbiological Specifications for Foods (ICMSF) (Pellissery et al., 2020). These results indicated that the low temperatures could inhibit the microbial activity, resulting in slowing down the microbial spoilage of beef during storage.

3.3 Effects of different storage temperatures on bacterial community structure of beef during storage

Comparison of bacterial community structure among three kinds of storage temperature at the phylum level

The bacterial relative abundance of 18 examined samples, namely Group C0 (0 d), Group C1 (1 d), Group C3 (3 d), Group C5 (5 d), Group C7 (7 d), Group C9 (9 d), Group S0 (0 d), Group S1 (1 d), Group S3 (3 d), Group S5 (5 d), Group S7 (7 d), Group S9 (9 d), Group F0 (0 d), Group F1 (1 d), Group F3 (3 d), Group F5 (5 d), Group F7 (7 d) and Group F9 (9 d), was analyzed by high-throughput sequencing technology. The bacterial community structure of Group C, Group S and Group F was analyzed at the phylum level as shown in Figure 1. Five bacterial phyla (abundance ≥ 1%), including Proteobacteria, Firmicutes, Bacteroidota, Actinobacteriota and Cyanobacteria were identified from Group C, Group S and Group F in initial of storage, in which the Proteobacteria was the primary dominant bacterial phylum with relative abundance of 72.72% followed by Bacteroidota and Firmicutes with relative abundance of 13.75% and 7.29%, respectively. The five bacterial phyla were detected through the whole three different storage conditions with a fluctuation in their relative abundance. In the Group S, the relative abundance of Firmicutes significantly increased during the superchiling storage ($p < 0.05$). In the Group F, the relative abundance of Actinobacteriota significantly increased during the frozen storage ($p < 0.05$).

Comparison of bacterial community structure among three kinds of storage temperature at the species level

Bacterial community structure of Group C, Group S and Group F was analyzed at the species level as shown in Figure 2. At the beginning of storage, the primary dominant bacterial species in beef samples was *Pseudomonas fragi* with

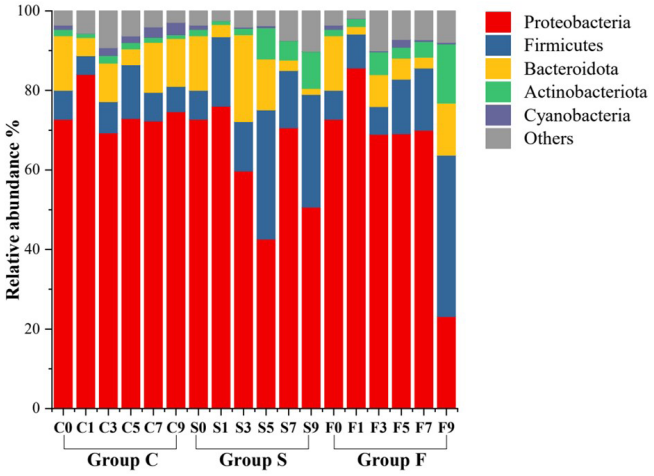

Figure 1. Relative abundance of bacteria community proportions at phylum level in Group C, Group S and Group F during three kinds of storage for 9 days.

Table 2. The total viable counts (TVC) of beef stored in three different temperatures condition for 7 days.

	Total viable counts (lg CFU/g)				
	0 d	1 d	3 d	5 d	7 d
Chilling storage	3.45 ± 0.05	4.12 ± 0.04	4.95 ± 0.01	6.27 ± 0.16	7.78 ± 0.06
Superchiling storage	3.08 ± 0.06	3.45 ± 0.014	3.76 ± 0.04	4.36 ± 0.01	4.82 ± 0.05
Frozen storage	3.11 + 0.06	3.13 ± 0.14	3.19 ± 0.01	3.24 ± 0.01	3.46 ± 0.02

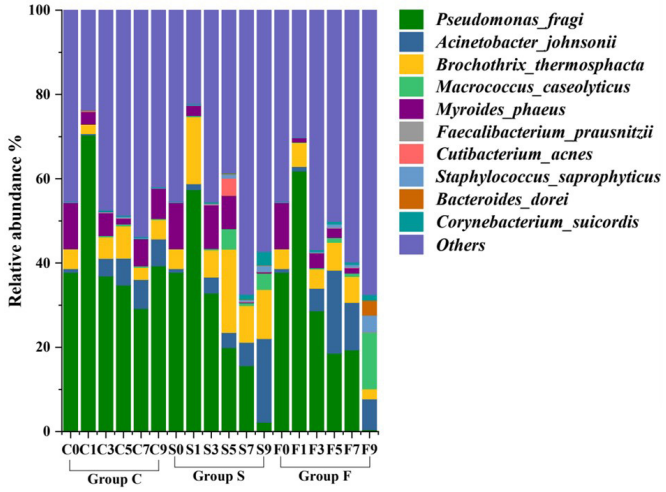

Figure 2. Relative abundance of bacteria community proportions at species level in Group C, Group S and Group F during three kinds of storage for 9 days.

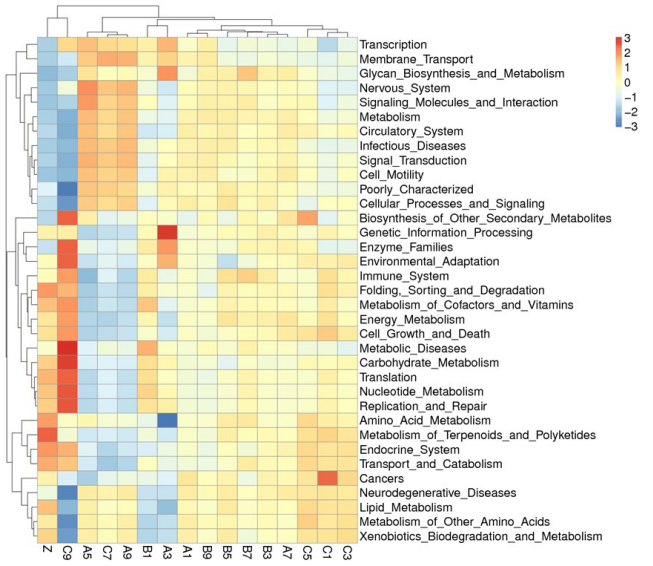

Figure 3. Abundance of functional properties related to microbial metabolism in fresh beef sample during three kinds of storage. Z: before storage, A: chilling storage (A1: 1 d, A3: 3 d, A5: 5 d, A7: 7 d, A9: 9 d), B: superchilling storage (B1: 1 d, B3: 3 d, B5: 5 d, B7: 7 d, B9: 9 d), C: frozen storage (C1: 1 d, C3: 3 d, C5: 5 d, C7: 7 d, C9: 9 d).

relative abundance of 37.78% followed by *Myroides phaeus* and *Brochothrix thermosphacta* with 10.95% and 4.64%, respectively. *P. fragi* has been recognized as the main spoilage bacteria for fresh meat in aerobic refrigeration, which can cause the physical damage, odor generation and mucus formation of meat (Sharma et al., 2009). Moreover, *P. fragi* can promote the growth of certain food borne pathogens, such as *Staphylococcus aureus* and *Listeria monocytogenes* (Marchand et al., 2009). As shown in Figure 2, on the first day of storage, the relative abundance of *P. fragi* significantly increased ($p < 0.05$) to 70.25%, 57.34%, 61.82% in Group C, Group S and Group F, repectively. On the 3rd day, the relative abundance of *P. fragi* significantly reduced ($p < 0.05$) to 34.71%, 32.82%, 28.61% in Group C, Group S and Group F, repectively. With the extension of storage time, the relative abundance of *P. fragi* in Group C maintained at a stable level, while the relative abundance of *P. fragi* in Group S and Group F significantly reduced ($p < 0.05$) to 2.21% and 0.42% on the 9th day, respectively. These results revealed that the growth of *P. fragi* cannot be inhibited in chilling storage, which may be one of the reasons for the fastest deterioration of the beef stored in chilling condition. In contrast to chilling storage, the growth of *P. fragi* was effectively inhibited under superchilling and frozen storage, suggesting that low temperature is conducive to inhibiting the growth of *P. fragi*. *M. phaeus* belongs to *Myroides phaeussp* spp., which is a low grade opportunistic pathogen and is often found in soil and water (Pérez-Lazo et al., 2020). In the Group C, the relative abundance of *M. phaeus* maintained a high level through the storage and was still 7.18% on the 9th day. In contrast to Group C, the relative abundance of *M. phaeus* remarkablely reduced in Group S and Group F, and *M. phaeus* was almost undetectable on the 9th day both in Group S and Group F. These results revealed that the low temperature is conducive to inhibiting the growth of *M. phaeus*. *Brochothrix thermosphacta* closely related to *Listeria* is nonpathogenic species and often isolated from meat and seafood products, which can cause spoilage by the production of off-odors (Illikoud et al., 2018). In all groups, the relative abundance of *M. phaeus* maintained a high level through the storage, suggesting that even the low temperature can not inhibit the growth of *B. thermosphacta*.

Moreover, *Acinetobacter johnsonii* belongs to *Acinetobacter spp.* and widely distributed in nature, which is a conditional pathogen and has been implicated in cases of meningitis (Zong & Zhang, 2013). In the raw beef, the existence of *A. johnsonii* was trace. However, with the extension of storage time, the relative abundance of *A. johnsonii* extremely increased in all groups, and reached to 6.37%, 19.81% and 6.93% in Group C, Group F and Group S, repectively. These results revealed that the *A. johnsonii* is a cold resistant bacteria. Thus, *A. johnsonii* should be paid special attention for meat duriing cold chain storage.

3.4 Analysis of functional characteristics of bacterial community

As shown in Figure 2, the dominant bacteria in beef stored in chilling, superchilling, and frozen storage was spoilage bacteria. The spoilage potential of bacteria depends on their ability to produce spoilage related metabolites. The functional characteristics of bacterial community of three groups during storage for 9 days were analyzed by heat map analysis as shown in Figure 3. Before storage, the higested relative abundance of genes was related to metabolism of terpenoids and polyketides followed by amino acid metabolism, lipid metabolism, folding-sorting and degradation, and transport and catabolism. For chilling storage, the enzyme families gradually increased and reached the maximum value on the 3rd day. In contrast to chilling storage, enzyme families level of samples stored in superchilling and frozen condition was lower. These results revealed that low temperature can well inhibit the bacterial enzyme activity, resulting in reduction of meat corruption. For the frozen storage, on the 9rd day, replication and

repair, carbohydrate metabolism and environmental adaption level was higher than other storage, suggesting that the bacteria activate their stress mechanism to survive in low temperature environment.

4 Conclusion

The storage temperature could significantly affect the microbial spoilage and bacterial community structure of fresh beef. Based on the quality factor analysis, superchilling storage is a good way to preserve freshness of beef. According to the analysis of bacterial community structure for samples stored in chilling, superchilling and frozen conditions, the growth of *P. fragi* was effectively inhibited under superchilling and frozen storage, which was conducive to slowing down thee microbial spoilage of beef during storage.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (31772093), and the Research Fund Project of Chengdu Agricultural College (20ZR110).

References

Cheng, J. R., Liu, X. M., & Zhang, Y. S. (2018). Characterization of Cantonese sausage fermented by a mixed starter culture. *Journal of Food Processing and Preservation*, 42(6), e13623. http://dx.doi.org/10.1111/jfpp.13623.

Ding, D., Zhou, C. Y., Ge, X. Y., Ye, K. P., Wang, P., Bai, Y., & Zhou, G. H. (2020). The effect of different degrees of superchilling on shelf life and quality of pork during storage. *Journal of Food Processing and Preservation*, 44(4), e14394. http://dx.doi.org/10.1111/jfpp.14394.

Holman, B. W. B., Mao, Y., Coombs, C. E. O., van de Ven, R. J., & Hopkins, D. L. (2016). Relationship between colorimetric (instrumental) evaluation and consumer-defined beef colour acceptability. *Meat Science*, 121, 104-106. http://dx.doi.org/10.1016/j.meatsci.2016.05.002. PMid:27294520.

Holman, B. W. B., van de Ven, R. J., Mao, Y., Coombs, C. E. O., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science*, 127, 57-62. http://dx.doi.org/10.1016/j.meatsci.2017.01.005. PMid:28130985.

Illikoud, N., Jaffrès, E., & Zagorec, M. (2018). Brochothrix thermosphacta. In T. M. Schmidt (Ed.), *Encyclopedia of microbiology* (4th ed., pp. 588-603). Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-0-12-809633-8.12106-5.

Kaur, M., Williams, M., Bissett, A., Ross, T., & Bowman, J. P. (2021). Effect of abattoir, livestock species and storage temperature on bacterial community dynamics and sensory properties of vacuum packaged red meat. *Food Microbiology*, 94, 103648. http://dx.doi.org/10.1016/j.fm.2020.103648. PMid:33279073.

Li, J., Shi, J., Huang, X., Zou, X., Li, Z., Zhang, D., Zhang, W., & Xu, Y. (2020). Effects of pulsed electric fifield on freeze-thaw quality of Atlantic salmon. *Innovative Food Science & Emerging Technologies*, 65, 102454. http://dx.doi.org/10.1016/j.ifset.2020.102454.

Li, N., Zhang, Y. X., Wu, Q., Gu, Q., Chen, M. T., Zhang, Y., Sun, X., & Zhang, J. (2019). High-throughput sequencing analysis of bacterial community composition and quality characteristics in refrigerated pork during storage. *Food Microbiology*, 83, 86-94. http://dx.doi.org/10.1016/j.fm.2019.04.013. PMid:31202422.

Liang, C., Zhang, D. Q., Zheng, X. C., Wen, X. Y., Yan, T. J., Zhang, Z. S., & Hou, C. L. (2021). Effects of different storage temperatures on the physicochemical properties and bacterial community structure of fresh lamb meat. *Food Science of Animal Resources*, 41(3), 509-526. http://dx.doi.org/10.5851/kosfa.2021.e15. PMid:34017958.

Mansur, A. R., Song, E. J., Cho, Y. S., Nam, Y. D., Choi, Y. S., Kim, D. O., Seo, D. H., & Nam, T. G. (2019). Comparative evaluation of spoilagerelated bacterial diversity and metabolite profiles in chilled beef stored under air and vacuum packaging. *Food Microbiology*, 77, 166-172. http://dx.doi.org/10.1016/j.fm.2018.09.006. PMid:30297047.

Marchand, S., Heylen, K., Messens, W., Coudijzer, K., Vos, P., Dewettinck, K., Herman, L., Block, J., & Heyndrickx, M. (2009). Seasonal influence on heat-resistant proteolytic capacity of pseudomonas lundensis and pseudomonas fragi, predominant milk spoilers isolated from belgian raw milk samples. *Environmental Microbiology*, 11(2), 467-482. http://dx.doi.org/10.1111/j.1462-2920.2008.01785.x. PMid:19196277.

National Health and Family Planning Commission of China. (2017). *National food safety standard: determination of volatile base nitrogen in foods (GB/T 5009.228-2016)*. Beijing: Standards Press of China.

Pan, C., Chen, S. J., Hao, S. X., & Yang, X. Q. (2019). Effect of low-temperature preservation on quality changes in Pacific white shrimp, *Litopenaeus vannamei:* a review. *Journal of the Science of Food and Agriculture*, 99(14), 6121-6128. http://dx.doi.org/10.1002/jsfa.9905. PMid:31260117.

Pellissery, A. J., Vinayamohan, P. G., Amalaradjou, M. A. R., & Venkitanarayanan, K. (2020). Spoilage bacteria and meat quality. In A. K. Biswas & P. K. Mandal (Eds.), *Meat quality analysis* (pp. 307-334). London: Academic Press. http://dx.doi.org/10.1016/B978-0-12-819233-7.00017-3.

Pérez-Lazo, G., Morales-Moreno, A., Soto-Febres, F., Jove-Químper, H., Morales-Castillo, L., Palomares-Reyes, C., Valle-Mendoza, J., Aguilar-Luis, M., & Silva-Caso, W. (2020). First report of myroides phaeus bacteraemia identified by polymerase chain reaction and genetic sequencing. *IDCases*, 19, e00695. http://dx.doi.org/10.1016/j.idcr.2020.e00695. PMid:32021799.

Pomponio, L., Bukh, C., & Ruiz-Carrascal, J. (2018). Proteolysis in pork loins during superchilling and regular chilling storage. *Meat Science*, 141, 57-65. http://dx.doi.org/10.1016/j.meatsci.2018.03.022. PMid:29605744.

Rumape, O., Elveny, M., Suksatan, W., Hatmi, R. U., Voronkova, O. Y., Bokov, D. O., & Wanita, Y. P. (2022). Study on the quality of fish products based on different preservation techniques: a review. *Food Science and Technology*, 42, e78521. http://dx.doi.org/10.1590/fst.78521.

Sharma, A., Bhattacharya, A., & Singh, S. (2009). Purification and characterization of an extracellular carbonic anhydrase from pseudomonas fragi. *Process Biochemistry*, 44(11), 1293-1297. http://dx.doi.org/10.1016/j.procbio.2009.07.022.

Tian, T., Kang, Y., Liu, L. J., & Wang, X. H. (2022a). The effect of superchilled preservation on shelf life and quality of beef during storage. *Food Science and Technology*, 42, e73222.

Tian, T., Liu, Y., & Wang, X. H. (2022b). Shelf-life extension of chilled beef by sodium lactate enhanced with Natamycin against discoloration and spoilage. *Food Science and Technology*, 42, e30522. http://dx.doi.org/10.1590/fst.30522.

Wang, X. H., Deng, Y. H., Sun, J. S., Ding, Y., Liu, Y., & Tian, T. (2022a). Unraveling characterizations of bacterial community and spoilage profiles shift in chilled pork during refrigerated storage. *Food Science and Technology*, 42, e80321. http://dx.doi.org/10.1590/fst.80321.

Wang, X. H., Ren, H. Y., Wang, W., Bai, T., & Li, J. X. (2015a). Evaluation of key factors influencing histamine formation and accumulation in

fermented sausages. *Journal of Food Safety*, 35(3), 395-402. http://dx.doi.org/10.1111/jfs.12187.

Wang, X., Ding, Y., Tian, T., & Liu, Y. (2022b). Comparative efficacy of sodium lactate and Natamycin against discoloration and spoilage of fresh beef during chilled storage. *Food Science and Technology*, 42, e74421. http://dx.doi.org/10.1590/fst.74421.

Wang, X., Ren, H. Y., Wang, W., Zhang, Y., Bai, T., Li, J. X., & Zhu, W. Y. (2015b). Effects of inoculation of commercial starter cultures on the quality and histamine accumulation in fermented sausages. *Journal of Food Science*, 80(2), M377-M383. http://dx.doi.org/10.1111/1750-3841.12765. PMid:25588615.

Wang, X., Ren, H., & Zhan, Y. (2018a). Characterization of microbial community composition and pathogens risk assessment in typical Italian-style salami by high-throughput sequencing technology. *Food Science and Biotechnology*, 27(1), 241-249. http://dx.doi.org/10.1007/s10068-017-0200-5. PMid:30263746.

Wang, X., Zhang, Y., Ren, H., & Zhan, Y. (2018b). Comparison of bacterial diversity profiles and microbial safety assessment of salami, Chinese dry-cured sausage and Chinese smoked-cured sausage by high-throughput sequencing. *LWT*, 90, 108-115. http://dx.doi.org/10.1016/j.lwt.2017.12.011.

Wang, X., Zhang, Y., Sun, J., Pan, P., Liu, Y., & Tian, T. (2021). Effects of starter culture inoculation on microbial community diversity and food safety of Chinese Cantonese sausages by high-throughput sequencing. *Journal of Food Science and Technology*, 58(3), 931-939. http://dx.doi.org/10.1007/s13197-020-04607-y. PMid:33678876.

Wen, Y. P., Kang, Y., Zhang, L., Tian, T., & Wang, X. H. (2022). Proteomics analysis to investigate the effect of sodium lactate on color stability of beef longissimus lumborum muscle during chilled storage. *Food Science and Technology*, 42, e55222. http://dx.doi.org/10.1590/fst.55222.

Zong, Z., & Zhang, X. (2013). Blandm-1-carrying acinetobacter johnsonii detected in hospital sewage. *The Journal of Antimicrobial Chemotherapy*, 68(5), 1007-1010. http://dx.doi.org/10.1093/jac/dks505. PMid:23288403.