Combined Effect of Plantaricin BM-1 and Refrigeration Storage to Control *Listeria Monocytogenes* Inoculated in Brined Cooked Ham

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Abstract—The effect of plantaricin BM-1 on the behavior of Listeria Monocytogenes in sliced brined cooked ham during refrigerated storage (4°Cand 9°C) was assessed. The combination of low storage temperature (4°C) and plantaricin can significantly reduced viable counts of L. Monocytogenes during storage (P < 0.05). When the same amount of plantaricin was applied, the growth of L. Monocytogenes in brined cooked ham stored at 4 °C was significantly slower than that in sample stored at 9°C (P < 0.01). Even combination of 1280 AU/g plantaricin and 4°C storage produced greater inhibition of L. monocytogenes than combination of 5120 AU/g plantaricin and 9°C. The combination of 4°C and 5120 AU/g plantaricin was the most effective treatment and can decrease the levels of L. monocytogenes 1.7 Log CFU/g at the end of storage.

Keywords-component; refrigerated storage; plantaricin; Listeria Monocytogenes; brined cooked ham

I. INTRODUCTION

Generally, ready-to-eat (RTE) meat products are free of pathogens after an adequate heat treatment during processing. However, they can easily be recontaminated by exposure to the environment during peeling, slicing, repackaging and other procedures [1,2]. *L. Monocytogenes* continues to be a major concern for food safety and its survival during the manufacture of brined cooked ham products is an important consideration [3,4]. Development of meat products with no added sodium nitrite could alter this sequence of hurdles and could have a negative effect on food safety. Therefore, the development of reduced salt meat products would require provide additional hurdles to pathogen growth in order to assure food safety and quality [5]. The application of bacteriocins as a preservation strategy has attracted increasing interest during recent years, as a result of the consumer demand for safe, fresh-tasting, RTE products with low levels of chemical preservatives during storage is the addition of natural antimicrobials as food ingredients [6,7]. Plantaricin BM-1 is produced by *Lactobacillus plantarum* BM-1, isolated from a traditionally fermented Chinese meat product. Plantaricin is very stable to heat and pH. We have proved in vitro that its broad bactericidal activity affects most of the Gram-positive bacteria and some Gram-negative bacteria [8].

The previous study had not been assessed the effect of plantaricin and refrigerated storage against L. Monocytogenes in sliced brined cooked ham without chemical preservative. Therefore, the present study aimed to assess the effects of plantaricin and storage temperature on the behavior of L. Monocytogenes on brined cooked ham during shelf life.

II. MATERIALS AND METHODS

A. Bacterial Strains and Culture Conditions

Lactobacillus plantarum BM-1, the bacteriocin producer strain was isolated from a traditionally fermented Chinese meat product [8]. The strain was cultured in MRS at 37 °C for 24 h and subcultured twice (2% inoculum). *L. monocytogenes* ATCC 54003 was used as a bacteriocin-sensitive strain to determine the inhibitory activity of plantaricin BM-1 preparations. Two consecutive pre-cultures in brain heart infusion (BHI, AoBoXing Company, Beijing, China) of 6 h and 12h, respectively.

B. Preparation of Plantaricin BM-1

3L sterile MRS broth was inoculated with 2% (V/V) of *L. plantarum* BM-1 and incubated at 37 °C. According to the method of Zhang et al. [8], partially purified plantaricin BM-1 was obtained. Before used, freeze-dried eluted fractions were dissolved in 1:40 of the initial culture volume in phosphate buffer (50 mM, pH 6.5) and sterilized by filtration through a 0.22um pore size Millex GP filter.

C. Assay for Bacteriocin Activity

Bacteriocin activity was determined by agar well diffusion method using stainless steel cylinders of 8 mm (outer) diameter and the bacteriocin-sensitive L. monocytogenes ATCC 54003 was used as the indicator strain. The extract titer was expressed in arbitrary units per ml (AU/ml). An arbitrary unit (AU) was defined as the highest dilution showing growth inhibition of the indicator lawn, and bacteriocin activity was expressed as AU/ml [9].

D. Brined Cooked Ham Manufacturing

Brined cooked ham was prepared with lean pork shoulder and the following additives (g/Kg) water, 200; sodium chloride, 26; dextrose, 0.6; sodium tripolyphosphate, 1.3; sodium hexametaphosphate, 1.3; sodium pyrophosphate, 1.3; sodium ascorbate, 0.35; sucrose, 10; potato starch, 30; soy protein isolated, 25. Pork shoulder meat bought from supermarket was minced in a cutter to a particle size of 3-4 mm and brined. Also, all the ingredients were homogenized in a mixer grinder for 30 min. The mixture was stuffed into 90 mm-diameter synthetic plastic casings and cooked by steam at 75 °C for 2h. Whole pieces of brined cooked ham were cooled at 1 °C for 12 h in a refrigerator and then aseptically sliced into 3-4 mm thick slices (approximately 25 g/slice).

E. Sample Preparation and Refrigerated Storage

Four independent lots were prepared: a control without plantaricin, three lots containing 1280, 2560, 5120 AU/g of plantaricin BM-1. Plantaricin preparation were added into meat paste to accordingly obtain three lots containing 1280, 2560, 5120 AU/g of plantaricin; Sliced brined cooked hams were inoculated with 10^4 CFU/g of *L. monocytogenes*. Afterwards, all brined cooked hams were vacuum-packaged with a packer in polyamide-polyethylene bags and stored at 4 °Cor 9 °C, respectively.

F. Enumeration of L. monocytogenes

Samples from each treatment were extracted in triplicate at selected times to determine viable counts of *L. Monocytogenes*. 25g ham were aseptically sampled and mixed (1:10) with dilution medium (0.1% peptone, 0.85% NaCl). Homogenisation was done in a stomacher for 2 min. After appropriate dilutions, enumeration of *L. Monocytogenes* performed by spread plating on PALCAM agar incubated at 37 °C for 48 h. The detection limit of the above techniques was 10 CFU/g. All microbial counts were expressed as lg (CFU/g).

G. Measurements of pH

The pH values were determined by homogenizing 10 g of ham sample in 90 mL distilled water (pH 7.0), with PHSJ - 5 Shanghai ray magnetic pH meter a Crison Basic 20 pH-meter.

H. Statistical Analyses

Statistical analyses was performed using the Analysis of Variance procedure of SAS Proprietary Software, version 9.0 (SAS Institute Inc., Cary, NC, USA.). Means was used to compare differences between treatments. The model included lot, storage temperature, storage time, and their interaction as fixed effects. Differences between effects were assessed by the Bonferroni t-test ($P < 0.05$).

III. Results and Analysis

A. Effect of Plantaricin BM-1 Combined with Refrigerated Storage on the L. monocytogenes Inoculated in Brined Cooked Ham

Refrigeration of sliced brined cooked ham at 4°C allowed the growth of *L. Monocytogenes in* control lot from inoculated levels (4.07 Log CFU/g) to a value of 8.45 log CFU/g at day 21 of storage (Fig. 1A). By contrast, refrigeration of ham at 9°C allowed the growth of *L. Monocytogenes in* control lot from inoculated levels (4.07 Log CFU/g) to a value of 8.91 log CFU/g at day 6 of storage (Fig. 1B). Thus, refrigeration at the low temperature proved to have a bacteriostatic effect against *L. monocytogenes*. However, it is unrealistic to maintaining 4°C throughout the distribution chain. This suggests the need to apply bacteriocins to chilled storage to prevent *L. Monocytogenes* growth.

Plantaricin BM-1 application significantly reduced the growth of *L. Monocytogenes* ($P < 0.05$) compared with control in brined cooked ham stored at 4 °C (Fig. 1A). The treatment with 5120 AU/g plantaricin was the most effective to control *L. monocytogenes* . *L. monocytogenes* counts in sample treated with 5120 AU/g plantaricin keep reducing from initial inoculums to 1.81 log CFU/g on day 21. Afterwards, *L. monocytogenes* were able to regrow, but viable counts for the samples were significantly lower ($P < 0.05$) than the control at the end of storage (Fig. 1A).

1280 AU/g plantaricin added to ham stored at 9 °C were not significantly effective in preventing the *L. monocytogenes* growth (Fig. 1B). 2560, 5120 AU/g of plantaricin BM-1 added to ham stored at 9 °C could significantly slow down($P < 0.05$)the breeding of *L. monocytogenes* compared to the control during whole storage period. Compared with the control, the greatest reduction of *L. monocytogenes* was observed in lot with 5120 AU/g of plantaricin.

When the same amount of plantaricin was applied, the growth of *L. Monocytogenes* in samples stored at 4 °C was significantly slower than that in sample stored at 9 °C ($P < 0.01$). Even the 1280 AU/g plantaricin and 4°C storage produced greater inhibition of *L. monocytogenes* than the 5120 AU/g plantaricin and 4°C treatment A1280 (Fig. 1).

B. pH Evolution

As shown in Fig.2, the pH values showed a rapid decrease from day 7 to day 14 in control samples, and remained stable in the further storage. Plantaricin BM-1 application significantly inhibited the decline of pH value compared with the control during whole storage ($p < 0.05$). For the samples added with 5120 AU/g plantaricin BM-1, a dramatic decrease ($P<0.05$).

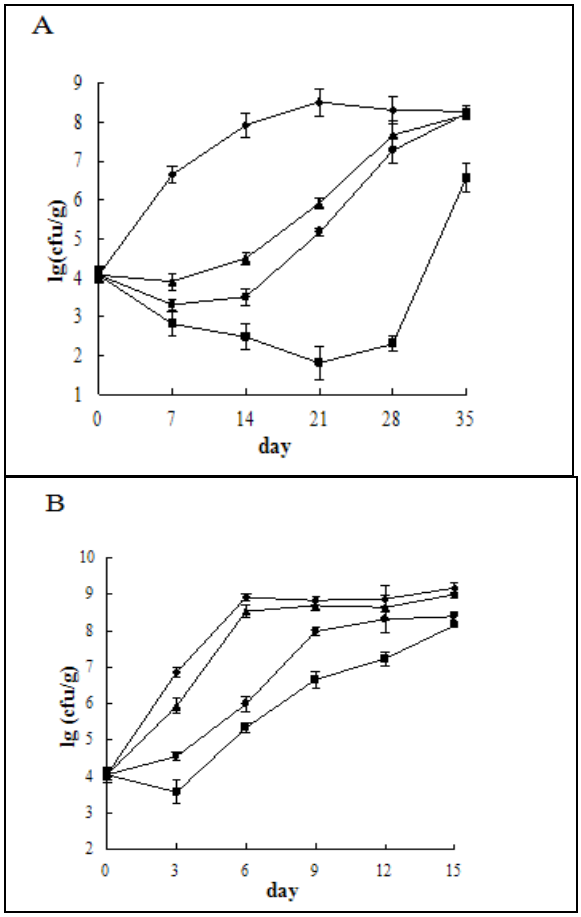

Figure 1. Evolution of *L. monocytogenes* (A, B) by plantaricin BM-1 at different concentrations in packaged sliced brined cooked ham without chemical preservative stored at 4 °Cor 9 °C. Control without plantaricin BM-1 (◆); Brined cooked ham treated with 1280 AU/g Plantaricin BM-1 (▲); Brined cooked ham treated with 2560 AU/g Plantaricin BM-1 (●); Brined cooked ham treated with 5120 AU/g Plantaricin BM-1 (■); Values are the average ± SD (error bars) of three individual packages of two independent experiments.

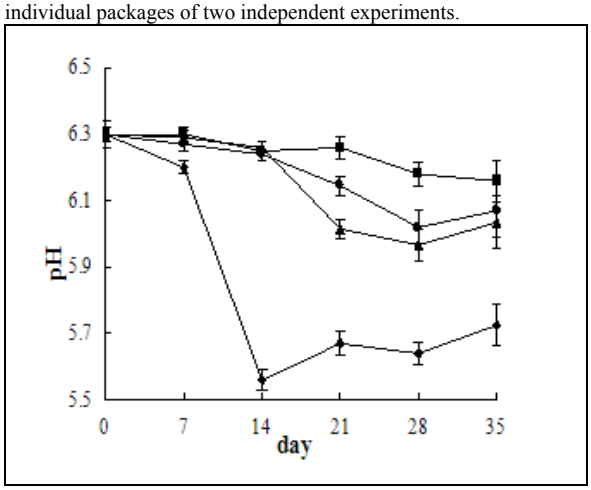

Figure 2. Evolution of pH by plantaricin BM-1 at different concentrations in packaged sliced brined cooked ham without chemical preservative stored at 4 °C. Control without plantaricin BM-1 (◆); Brined cooked ham treated with 1280 AU/g Plantaricin BM-1 (▲); Brined cooked ham treated with 2560 AU/g Plantaricin BM-1 (●); Brined cooked ham treated with 5120 AU/g Plantaricin BM-1 (■); Values are the average ± SD (error bars) of three individual packages of two independent experiments.

IV. Discussion

Although *L. Monocytogenes* is able to grow during refrigerated storage, temperature control has proved to be essential in reducing the risk of the pathogen. At 4°Cthe growth of *L. Monocytogenes* was slower than at 9°C and the counts were between 1.54 and 4 Log CFU/g lower than at 6°C on day 15. Thus, refrigeration at the low temperature proved to have a bacteriostatic effect against *L. monocytogenes.* A similar observation has been made by Anna Jofre et al [10], who reported that at 1°C the growth of *L. Monocytogenes* was slower than at 6°C and the counts reached by the end of storage were between 1.4 and 7.2 Log CFU/g lower than at 6°C. When the same amount of plantaricin was applied, the growth of *L. Monocytogenes* in samples stored at 4 °C was significantly slower than that in sample stored at 9 °C ($P < 0.01$). 5120AU/g plantaricin applied as an additional hurdle to refrigeration temperature (4°C) was effective for controlling *L. monocytogenes* at the end of storage, which 1.7 log CFU/g lower than control.

V. Conclusions

In conclusion, the results presented here show that addition of plantaricin BM-1 in the formulation of brined cooked ham and storage at 4°Cprovides an effective protection against *L. monocytogenes*. Especially, The combination of 4 °C and 5120 AU/g plantaricin was the most effective treatment to control and can decrease the levels of *L. monocytogenes* 1.7 Log CFU/g at the end of storage.

Acknowledgements

The research was financially supported by National Program Grant from the Ministry of Science and Technology of the People's Republic of China (No.SS2012AA101606-05), the Importation and Development of High-Caliber talents project of Beijing Municipal Institutions (CIT&TCD20140315).

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