THE ROLE OF BACTERIAL FERMENTATION IN LIPOLYSIS AND LIPID OXIDATION IN HARBIN DRY SAUSAGES

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Abstract – *Pediococcus pentosaceus, Lactobacillus curvatus, Lactobacillus sake* and *Staphylococcus xylosus* were evaluated to determine their roles in the lipolysis and lipid oxidation in Harbin dry sausages and their relation to flavour development. The free fatty acid contents of both muscle and fat tissues were higher in the inoculated sausages than those of the non-inoculated control, especially with mixed strains ($P<0.05$). The inoculation of dry sausages with bacterial strains, especially mixed strains, significantly decreased the peroxide value and thiobarbituric acid reactive substances ($P<0.05$). The results demonstrate that Harbin dry sausage can be inoculated with a starter culture mixture of *P. pentosaceus, L. curvatus* and *S. xylosus* to promote lipid hydrolysis and inhibit lipid autoxidationt.

Key Words – Fermented sausages, Free fatty acids, Lactic acid bacteria, *Staphylococcus xylosus*

I. INTRODUCTION

Lipids in dry sausages are located in muscle tissue and fat tissue. Pork back fat consists mainly of triglycerides, while lean meat consists of approximately 7% lipids. The lipids in muscle tissue are intramuscular fat, which consists of 62%-80% triglycerides and 16%-34% phospholipids [1]. Although phospholipids are present in lower amounts than triglycerides, they are more susceptible to lipolysis and oxidation because they are rich in polyunsaturated fatty acids (PUFA) [2]. FFAs are the main substrates for lipid oxidation; therefore, lipolysis is a critical step for flavour development. Although endogenous enzymes, such as lipases and phospholipases, are considered to be the main causes of free fatty acid (FFA) release, bacterial lipase activity cannot be neglected [3]. Coagulase-negative Staphylococci (CNS) and lactic acid bacteria (LAB) are the most common microorganisms found in fermented meats and have been used as starter cultures in fermented meats [4-6]. Lipases have been extracted, purified and characterised in *Lactobacillus plantarum*, *Staphylococcus xylosus* and *Staphylococcus warneri*. Furthermore, lipolytic activity of these microorganisms has been detected in fermented meat products, especially in *Staphylococcus* strains [7].

Moreover, moderate oxidation of FFAs derived from lipolysis plays a significant role in flavour development [8]. Volatile compounds such as linear aldehydes, ketones, corresponding alcohols, acids and esters were generated from FFA autoxidation. However, excessive lipid oxidation is the main cause of quality deterioration in fermented meat products, resulting in discolouration, drip loss, rancidity, loss of nutrient value, and meat protein oxidation [9]. Therefore, the inoculation of bacterial strains with the ability to inhibit lipid oxidation is a novel method of improving the development of the fermented flavour. Several studies have demonstrated the antioxidant potential of CNS and LAB *in vitro* and in meat product models [6, 7, 10].

The objective of the present study was to assess the potential role of four bacterial strains isolated from Harbin dry sausages in the lipolysis of both muscle and fat tissues and oxidation in Harbin dry sausages. Total FFA contents were analysed to evaluate the degree of lipolysis. To determine the level of oxidation, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were detected.

II. MATERIALS AND METHODS

1. Bacterial cultures and growth media

Three LAB strains and one *Staphylococcus* strain were used in this study. These strains included *P. pentosaceus* R1, *L. curvatus*, *L. sake* and *Staphylococcus xylosus* A1. *P. pentosaceus* R1 and *S. xylosus* A1 were previously isolated from Harbin dry sausages and identified by 16S

rDNA sequencing according to Zhao *et al* [11, 12]. *L. curvatus* and *L. sake* were bought from the China General Microbiological Culture Collection Centre. *P. pentosaceus* R1, *L. curvatus* and *L. sake* were kept on de Man-Rogosa-Sharpe (MRS) agar plates, and *S. xylosus* A1 was kept on Mannitol Salt Agar (MSA) plates at 4 °C until use.

2. Preparation of Harbin dry sausage

Seven batches of dry sausages were manufactured. A control batch was not inoculated with starter culture, and the other batches were inoculated with various single strains or mixed strains. Single strains included *P. pentosaceus* R1 (Pp), *L. curvatus* (Lc), *L. sake* (Ls) and *S. xylosus* A1 (Sx), respectively. One batch of mixed strains was composed of *P. pentosaceus* R1, *S. xylosus* A1 and *L. curvatus* (Pp+Sx+Lc), and another was composed of *P. pentosaceus* R1, *S. xylosus* A1 and *L. sake* (Pp+Sx+Ls).Sausages were prepared according to the method of Chen *et al*. [10].

3. Measurement of lipid hydrolysis

The lipid hydrolysis of both muscle and fat tissues in dry sausages was assessed by measuring total FFA content. The FFA extraction was carried out according to the method of Folch *et al* [13]. The total content of FFAs was measured as described by Natseba *et al* [14]. The FFA concentration is expressed in oleic acid equivalents: FFA (mg/g lipid) = N × V × M/W, where N is the standard concentration of NaOH (M), V is the consumed volume of NaOH (mL), M is the molecular weight of oleic acid (280.2 g/mol), and W is the sample weight (g).

4. Measurement of lipid oxidation

The lipid oxidation level in dry sausages was evaluated by PV and TBARS. The PV of dry sausages was determined according to the method of Vareltzis *et al* [15]. The lipid PV is expressed as meq/kg of lipid.

The TBARS of dry sausages were detected according to the method of Wang *et al* [16]. The TBARS value, expressed as mg of malonaldehyde/kg of the sausage, was calculated using the following equation: TBARS (mg/kg meat) = $(A_{532} / W_s) \times 9.48$, where A_{532} is the absorbance (532 nm) of the assay solution, W_s is the sausage weight (g), and "9.48" is a constant derived from the dilution factor and the molar extinction coefficient (152,000 M^{-1} cm^{-1}) of the red thiobarbituric acid reaction product.

5. Statistical analysis

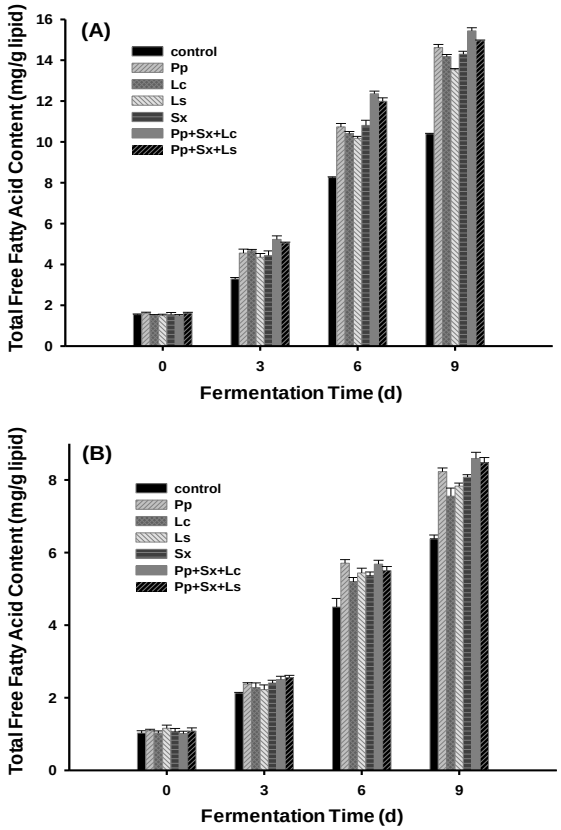
All the data were analyzed statistically using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St Paul, MN, USA), and presented as mean ± standard deviations (SD). One-way analysis of variance (ANOVA) with the Tukey's multiple comparison was used to measure the significance of the main effects ($P < 0.05$).

III. RESULTS AND DISCUSSION

1. Lipid hydrolysis

As shown in Fig. 1, the total FFA concentrations of both muscle and fat tissues increased gradually ($P < 0.05$). Initially, the total FFA concentration in muscle tissue was 1.54 mg/g lipid. After 9 d of fermentation, the total FFA concentration of muscle tissue increased to 10.39, 14.62, 14.18, 13.54, 14.30, 15.44 and 14.95 mg/g lipid from sausages with non-inoculated starter and inoculated starter cultures of Pp, Lc, Ls, Sx, Pp+Sx+Lc and Pp+Sx+Ls. There was a similar result in fat tissue, where the total FFA concentration of fat tissue rose from 1.07 mg/g lipid to 6.38, 8.23, 7.55, 7.83, 8.08, 8.59 and 8.47 mg/g lipid in sausages without starter culture and with starter cultures of Pp, Lc, Ls, Sx, Pp+Sx+Lc and Pp+Sx+Ls, respectively. The total FFA concentrations of the lipids in muscle tissue were higher than those in fat tissue over the course of the fermentation ($P < 0.05$). Additionally, the total FFA concentrations in the inoculated sausages were higher than those in the controls ($P < 0.05$), especially in sausages inoculated with starter cultures Pp+Sx+Lc and Pp+Sx+Ls. The increased total FFA concentration could be ascribed to the bacterial lipolytic activity. The synergistic actions of endogenous enzymes, bacterial lipolytic activities and suitable reaction conditions can accelerate lipid hydrolysis.

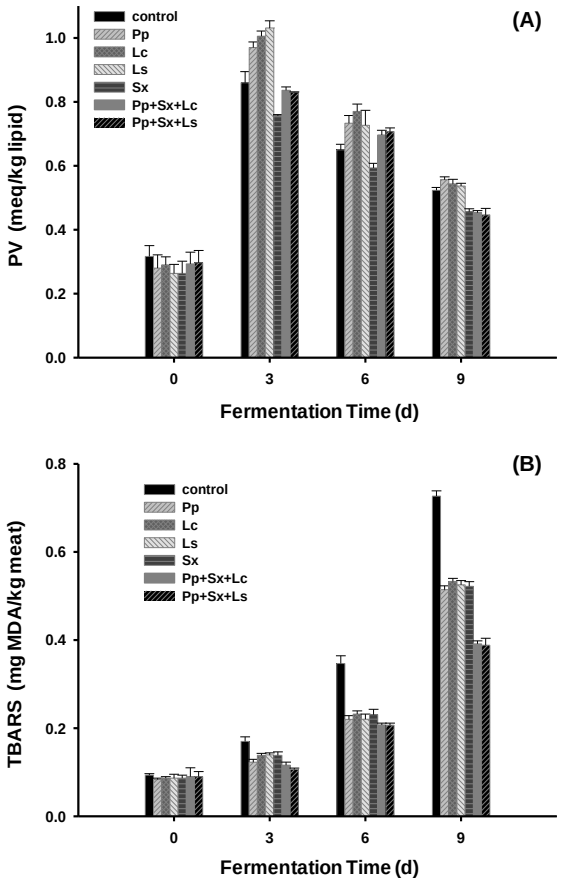
Figure 1. Total free fatty acid contents (mg/g lipid) of muscle (A) and fat tissue (B) over a nine-day fermentation in Harbin dry sausages non-inoculated and inoculated with various bacterial strains.



2. Lipid oxidation

Lipid oxidation of dry sausages was evaluated by monitoring PV and TBARS over the course of fermentation. As shown in Fig. 2 (A), the initial PV of all sausages was 0.29 meq/kg lipid, which sharply increased to its highest level of 0.83-1.03 meq/kg lipid at day 3 and then decreased ($P < 0.05$), indicating that the rate of formation of hydroperoxides was slower than that of their decomposition. Additionally, the PV in the sausages inoculated with *P. pentosaceus*, *L. curvatus* and *L. sake* was higher than that in the other sausages ($P < 0.05$). It has been reported that hydrogen peroxide is produced by LAB metabolism, which also caused the PV to increase. A lower PV was detected in the inoculated sausages with a starter culture containing *S. xylosus* ($P < 0.05$), which due to the antioxidant action of *S. xylosus*. Several antioxidant enzymes, such as catalase, have been detected in *S. xylosus*, which can decompose hydrogen peroxide into water [7].

Figure 2. Peroxide value (PV) (meq/kg lipid) (A) and thiobarbituric acid reactive substances (TBARS) (mg MDA/kg meat) (B) over a nine-day fermentation in Harbin dry sausages non-inoculated and inoculated with various bacterial strains.



TBARS presented a constant increase in all sausages during fermentation and remained at a much higher amount in the control at day 6 and day 9 ($P < 0.05$). As shown in Fig. 2 (B), the TBARS in the control were 35.7%, 33.4%, 33.2%, 34.8%, 51.1% and 51.4% higher at the end of fermentation than those in the sausages inoculated with starter Pp, Lc, Ls, Sx, Pp+Sx+Lc and Pp+Sx+Ls, respectively ($P < 0.05$). These findings showed that lipid oxidation was inhibited by bacterial fermentation. Moreover, the antioxidant effect of *P. pentosaceus* on lipid oxidation in fermented sausages has been proven in one of our previous studies [10].

IV. CONCLUSION

The effect of bacterial fermentation in the lipolysis and lipid oxidation in Harbin dry sausages was determined in our study. Total FFA concentration indicated that the inoculation of bacterial strains, especially multiple strains, promotes the hydrolysis of lipids in both muscle and fat tissues. Moreover, the lipolysis in muscle tissue was more severe than that in fat tissue. The analyses of PV and TBARS showed that bacterial fermentation can inhibit lipid oxidation. Therefore, fermentation of dry sausages with multiple bacterial strains can promote lipid hydrolysis and inhibit lipid autoxidation.

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REFERENCES

1. Olivares, A., Navarro, J. L., & Flores, M. (2009). Distribution of volatile compounds in lean and subcutaneous fat tissues during processing of dry fermented sausages. Food Research International, 42, 1303-1308.
2. Fuentes, V., Estévez, M., Ventanas, J., & Ventanas, S. (2014). Impact of lipid content and composition on lipid oxidation and protein carbonylation in experimental fermented sausages. Food Chemistry, 147, 70-77.
3. Molly, K., Demeyer, D., Johansson, G., Raemaekers, M., Ghistelinck, M., & Geenen, I. (1997). The importance of meat enzymes in ripening and flavour generation in dry fermented sausages. First results of a European project. Food Chemistry, 59, 539-545.
4. Bedia, M., Méndez, L., & Bañón, S. (2011). Evaluation of different starter cultures (*Staphylococci* plus Lactic Acid Bacteria) in semi-ripened Salami stuffed in swine gut. Meat Science, 87, 381-386.
5. Dalmış, Ü., & Soyer, A. (2008). Effect of processing methods and starter culture (*Staphylococcus xylosus* and *Pediococcus pentosaceus*) on proteolytic changes in Turkish sausages (*sucuk*) during ripening and storage. Meat Science, 80, 345-354.
6. Leroy, F., Verluyten, J., & Vuyst, L. D. (2006). Functional meat starter cultures for improved sausage fermentation. International Journal of Food Microbiology, 106, 270-285.
7. Flores, M., & Toldrá, F. (2011). Microbial enzymatic activities for improved fermented meats. Trends in Food Science & Technology, 22, 81-90.
8. Zanardi, E., Ghidin, S., Battaglia, A., & Chizzolini, R. (2004). Lipolysis and lipid oxidation in fermented sausages depending on different processing conditions and different antioxidants. Meat Science, 66, 415-423.
9. Zanardi, E., Ghidin, S., Battaglia, A., & Chizzolini, R. (2004). Lipolysis and lipid oxidation in fermented sausages depending on different processing conditions and different antioxidants. Meat Science, 66, 415-423.
10. Chen, Q., Kong, B., Sun, Q., Dong, F., & Liu, Q. (2015). Antioxidant potential of a unique LAB culture isolated from Harbin dry sausage: *In vitro* and in a sausage model. Meat Science, 110, 180-188.
11. Zhao, J., & Kong, B. (2010a). Isolation and of lactic acid bacteria from Chinese-style naturally dry fermented sausages. Science and Technology of Food Industry, 34(158-160), 324.
12. Zhao, J., & Kong, B. (2010b). Screening and identification of *Staphylococci* from the Chinese-style naturally dry fermented sausages. China Brewing, 10, 50-53.
13. Folch, J., Lees, M., & Sloane-Stanley, G. A. (1957). Simple method for the isolation and purification of total lipids from animal tissues. The Journal of Biological Chemistry, 226, 497-509.
14. Natseba, A., Lwalinda, I., Kakura, E., Muyanja, C. K., & Muyonga, J. H. (2005). Effect of pre-freezing icing duration on quality changes in frozen Nile perch (*Lates niloticus*). Food Research International, 38, 469-474.
15. Vareltzis, P., Hultin, H. O., & Autio, W. R. (2008). Hemoglobin-mediated lipid oxidation of protein isolates obtained from cod and haddock white muscle as affected by citric acid, calcium chloride and pH. Food Chemistry, 108, 64-74.
16. Wang, L. L., & Xiong, Y. L. (2005). Inhibition of lipid oxidation in cooked beef patties by hydrolyzed potato protein is related to its reducing and radical scavenging ability. Journal of Agricultural and Food Chemistry, 53, 9186-9192.