LWT - Food Science and Technology 77 (2017) 389–396

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development

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ARTICLE INFO

Article history:
Received 8 June 2016
Received in revised form
24 November 2016
Accepted 27 November 2016
Available online 29 November 2016

Keywords:
Dry sausage
Bacterial fermentation
Lipid hydrolysis
Lipid oxidation
Volatile compounds

ABSTRACT

Pediococcus pentosaceus, *Lactobacillus curvatus*, *Lactobacillus sakei* 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 (FFA) 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 predominant FFAs in both tissues at the end of the fermentation were palmitic acid, stearic acid and oleic acid. The inoculation of dry sausages with bacterial strains, especially mixed strains, significantly decreased the peroxide value and thiobarbituric acid reactive substances ($P < 0.05$). Furthermore, a lower level of volatile compounds related to lipid-oxidation, such as aldehydes, ketones and hydrocarbons, was observed in the inoculated sausage ($P < 0.05$). 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, inhibit lipid autoxidation and improve fermented flavour development.

1. Introduction

Harbin dry sausage is a naturally fermented traditional Chinese sausage with a short production cycle (approximately 15 d), distinctive texture, and special fermented flavour. It is manufactured with 90% minced lean pork and 10% pork back fat (Chen, Kong, Sun, Dong, & Liu, 2015). Throughout its fermentation, lipid hydrolysis and oxidation in muscle and fat tissues are important processes contributing to the development of the fermented flavour. Free fatty acids (FFAs) are released from lipids by the synergistic action of endogenous enzymes and bacterial lipolytic enzymes (Flores & Toldrá, 2011; Leroy, Verluyten, & Vuyst, 2006). Some FFAs with short chains (<6 C) directly contribute to the presence of strong cheesy odours, while medium and long chain FFAs are further subjected to oxidation, leading to the generation of additional volatile compounds (Ansorena, Gimeno, Astiasarán, & Bello, 2001).

Lipids in dry sausages are located in muscle tissue (lean pork) and fat tissue (pork back fat). 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 (Olivares, Navarro, & Flores, 2009). 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) (Fuentes, Estévez, Ventanas, & Ventanas, 2014; Gómez & Lorenzo, 2013). 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 (Molly et al., 1997). 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 (Bedia, Méndez, & Bañón, 2011; Dalmış & Soyer, 2008; Xu, Xia, Yang, Kim, & Nie, 2010; Leroy et al., 2006; Lorenzo, Gómez,& Fonseca, 2014). 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 (Flores & Toldrá, 2011).

Moreover, moderate oxidation of FFAs derived from lipolysis plays a significant role in flavour development (Lorenzo, Gómez,

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http://dx.doi.org/10.1016/j.lwt.2016.11.075
0023-6438/

Purriños, & Fonseca, 2016; Qiu, Zhao, Sun, Zhou, & Cui, 2013; Zanardi, Ghidin, Battaglia, & Chizzolini, 2004). Volatile compounds such as linear aldehydes, ketones, corresponding alcohols, acids and esters were generated from FFA autoxidation. In general, five-to nine-carbon aldehydes can provide the oil and lipid odours of fermented meats, and long chain aldehydes are related to a laurel odour. For example, hexanal, which is a typical product of linoleic acid oxidation, contributes to a fresh cut grass odour; heptanal contributes to a nut odour; and nonanal contributes to the citrus, laurel and carnation odours (Xu et al., 2014). Additionally, methyl ketone originating from microbial incomplete β-oxidation is one of the main contributors to a characteristic fermented flavour. 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 (Falowo, Fayemi, & Muchenje, 2014; Zanardi et al., 2004). Therefore, the inoculation of bacterial strains with the ability to inhibit lipid oxidation is a good 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 (Chen et al., 2015; Flores & Toldrá, 2011; Leroy et al., 2006).

In previous studies, we isolated and identified the main microorganisms in Harbin dry sausage, including 11 strains of *Staphylococcus*, 14 strains of LAB, and 10 strains of yeast (Zhao & Kong, 2010a,b,c). Our results revealed that *Pediococcus pentosaceus*, *Lactobacillus curvatus*, *Lactobacillus sakei*, and *S. xylosus* resulted in proteolysis and flavour formation in pork muscle sarcoplasmic proteins (Chen, Liu, Sun, Kong, & Xiong, 2015). In addition, we found that *P. pentosaceus* had significant antioxidant potential when used as the starter culture for dry sausages (Chen et al., 2015). We also evaluated role of those four strains in the hydrolysis and oxidation of sarcoplasmic and myofibrillar proteins in Harbin dry sausages. The results showed that bacterial fermentation, especially fermentation with multiple strains, promotes the hydrolysis of meat proteins. Analysis of carbonyl and total sulfhydryl contents and surface hydrophobicity showed that bacterial fermentation can delay protein oxidation. Meanwhile, a sensorial analysis has been carried out, and results showed that the sausages inoculated with multiple strains were more favoured by panelists (Chen, Kong, Han, Liu, & Xu, 2016). 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 and FFA composition were analysed to evaluate the degree of lipolysis. To determine the level of oxidation, peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and volatile compounds were detected.

2. Materials and methods

2.1. Materials and chemicals

Lean pork and pork back fat (centre temperature was 4 °C) were purchased less than 24 h post-mortem from Beidahuang Meat Processing Co. (Harbin, China) and kept on ice during transport to the meat science laboratory at Northeast Agricultural University. Trichloroacetic acid (TCA) and 2,6-ditert-butyl-methylphenol (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were purchased from Solabio Corporation (Beijing, China).

2.2. 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. sakei* and *S. xylosus* A1. *P. pentosaceus* R1 and *S. xylosus* A1 were previously isolated from Harbin dry sausages and identified by 16 S rDNA sequencing according to Zhao and Kong (2010a,b). *L. curvatus* and *L. sakei* were bought from the China General Microbiological Culture Collection Centre. *P. pentosaceus* R1 and *S. xylosus* A1 have been proved to be beneficial to flavour and colour formation in our previous studies (Chen et al., 2015; Li, Kong, Chen, Zheng, & Liu, 2013), and both strains are essential in the mixed starter cultures. As lactobacillus, *L. curvatus* and *L. sake* possess a stronger acid-producing activity, which are commonly used in fermented meats to accelerate the acidification. *P. pentosaceus* R1, *L. curvatus* and *L. sakei* 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. MRS broth was prepared according to Chen et al. (2015). MSA broth was prepared with beef extract (1.0 g/L), peptone (10.0 g/L), D-mannitol (10.0 g/L), and sodium chloride (75.0 g/L) at a final pH of 7.4 ± 0.2.

2.3. 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. sakei* (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. sakei* (Pp + Sx + Ls).

Sausages were prepared according to the method of Chen et al. (2015). Cell pellets of strains were diluted with isotonic saline to create cell suspensions. Meat, fat and additives were mixed thoroughly in a mixer. The meat batter was then inoculated with cell suspension to a final concentration of 10^7 CFU/g meat according to our previous results (not published). The control batch was not inoculated. The meat batter was thoroughly mixed and stuffed into natural porcine casings of 3.0 cm in diameter to produce sausages with final weights of approximately 0.15 kg. All batches of sausages were air-dried at 25 ± 2 °C for 1 d (30%–50% relative humidity) and then transferred to an incubator at 25 ± 2 °C and 75%–80% relative humidity for fermentation. After a nine-day fermentation, Harbin dry sausage was cooked, and then vacuum packaged. At various times (0, 3, 6, and 9 d), three sausages from each batch were used to analyse lipid hydrolysis, lipid oxidation and volatile compounds.

2.4. Measurement of lipid hydrolysis

The lipid hydrolysis of both muscle and fat tissues in dry sausages was assessed by measuring total FFA content and composition. The muscle and fat tissues in dry sausage were separated and minced for analysis.

2.4.1. FFA extraction

According to the method of Folch, Lees, and Sloane-Stanley (1957) with some modifications, sample (20.0 g) diluted with twenty volumes (w/v) of an ice-cold chloroform-methanol (2:1, v/v) mixture was homogenised for 3 min in a Polytron homogeniser (IKA T18 basic, IKA-Werke GmbH & Co., Staufen, Germany), and then the extracted solution was filtered and concentrated on a rotary vacuum evaporator (Eyela N-1000, Tokyo Rikakikai Co. Ltd., Tokyo, Japan). The extraction of FAAs was conducted in NH_2-aminopropyl mini-columns (500 mg/6 mL, Phenomenex, USA) using 3.0 mL of an acetic acid-ethylether mixture by the method of Kaluzny, Duncan, Merritt, and Epps (1985) with some modification.