Meat Science 98 (2014) 34–40

Contents lists available at ScienceDirect

Meat Science

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

# Effect of Flavourzyme on proteolysis, antioxidant capacity and sensory attributes of Chinese sausage

Li Feng, Yan Qiao, Yufeng Zou, Ming Huang *, Zhuangli Kang, Guanghong Zhou

*Nanjing Innovation Center of Meat Products Processing, Synergetic Innovation Center of Food Safety and Nutrition, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China*

ARTICLE INFO

*Article history:*
Received 2 November 2013
Received in revised form 5 April 2014
Accepted 7 April 2014
Available online 18 April 2014

*Keywords:*
Chinese sausage
Flavourzyme
Proteolysis
Antioxidant capacity
Texture profile analysis
Sensory attributes

ABSTRACT

The objective of this study was to investigate the effect of Flavourzyme, at levels of 0 (control) 4, 8, 12, 16 and 20 LAPU/kg raw meat, on the proteolysis, antioxidant capacity and sensory attributes of Chinese sausage made at 50 °C for 48 h. Results showed that Flavourzyme addition in Chinese sausage accelerated protein degradation, which was reflected by the increase of non-protein nitrogen and appearance of new protein bands in both water-soluble and salt-soluble proteins. By adding Flavourzyme, texture profile analysis (TPA) parameters decreased significantly, and aroma, taste and texture scores were enhanced, respectively. The best sensory attributes were obtained at 8 and 12 LAPU/kg Flavourzyme dose. Besides, Flavourzyme addition enhanced antioxidant capacity, lowered water activity and TBARS values of Chinese sausage. Therefore, moderate Flavourzyme addition is a novel method with great potential to improve eating properties and storage stability of Chinese sausage.



## 1. Introduction

Traditional Chinese sausage is famous for its unique cured meat flavor that comes from natural fermentation for long duration in winter (Wang, Jiang, & Lin, 1995). Since natural maturation is time consuming and climate limited, recently some alternative methods have been used by most commercial manufactures to shorten the process time. However, these alternative methods induce the problems associated with flavor deficiency and tough texture (Lin & Huang, 2008). Among the alternative ways, high temperature dehydration procedure (45–65 °C) has been the most popular choice (Zhang, Lin, Leng, Huang, & Zhou, 2013). However, high temperature can accelerate lipid oxidation (Lin & Liang, 2002), leading to the deterioration of finished products.

Thus, enhancing flavor, softening texture and preventing excessive lipid oxidation are ways that commercial manufactures could improve the eating and storage qualities of Chinese sausage.

Proteolysis is considered to be one of the most important biochemical changes during the ripening of fermented sausages (Díaz, Fernandez, De Fernando, de la Hoz, & Ordoñez, 1997; García de Fernando & Fox, 1991; Hughes et al., 2002) and dry-cured meat products (Fanco, Prieto, Cruz, López, & Carballo, 2002; Lorenzo, Garcı'a Fontán, Franco, & Carballo, 2008; Toldra', 1998). Proteolysis has been proved to be a critical process in texture softening (Benito, Rodríguez, Córdoba, Andrade, & Córdoba, 2005) and generation of meat flavor (Toldra', 1998) in fermented sausages. It has been found that, proteolysis is catalyzed not only by endogenous enzymes, such as cathepsins and trypsin-like peptidases, but also by proteases produced by microorganisms involved in the ripening process (Benito, Rodríguez, Martín, Aranda, & Córdoba, 2004). Protein hydrolysis during process of fermented meat products involves a degradation of high molecular weight proteins, resulting in the formation of polypeptides and free amino acids (Sun et al., 2009). Considering that the amount of endogenous enzymes are limited and those endogenous enzymes may be inhibited by drying temperature and curing agents during the manufacturing process, appropriate exogenous proteases seems to be more effective in accelerating proteolysis of Chinese sausage. It has been reported that the use of exogenous protease leads to the acceleration of proteolysis, sausage tenderization (Benito et al., 2004, 2005), sensory enhancement (Benito et al., 2004) and inhibiting lipid oxidation (Broncano, Timón, Parra, Andrés, & Petrón, 2011) of dry fermented sausages. This means adding exogenous proteases may be a potential method to significantly improve the qualities of Chinese sausage.

Flavourzyme is a mixture of proteases with both exo- and endopeptidase activities, obtained by the controlled fermentation of *A. oryzae*. Flavourzyme has been reported to change volatile compounds profile of dry fermented sausage (Ansorena, Astiasaran, & Bello, 2000) by accelerating proteolysis. To the best of our knowledge, there were few

* Corresponding author at: College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China. Tel.: +86 25 84396808; fax: +86 25 84395939.
*E-mail address:* mhuang@njau.edu.cn (M. Huang).

http://dx.doi.org/10.1016/j.meatsci.2014.04.001
0309-1740/

studies dealing with the use of Flavourzyme on proteolysis, antioxidant capacity and sensory qualities in cured meat products ripened at high temperature (50 °C) like Chinese sausage. Therefore, the aim of the present study was to investigate the effect of different levels of Flavourzyme (a commercial protease) on proteolysis, antioxidant capacity and sensory attributes in Chinese sausage.

## 2. Materials and methods

### 2.1. Sausage preparation and sampling

Fresh boneless chilled pork lean pork and back fat were purchased from a local commercial meat processing company (Sushi Group, Jiangsu, China) in which pigs were slaughtered according to the requirements of National Standards of China "Pig Slaughter and Quarantine Regulations". Natural salt hog casings were obtained from Far-Eastern Casing Co., Ltd., Hebei, China. Flavourzyme was obtained from Novozymes China Inc. (Jiangsu, China). The lean pork were ground through a 5 mm plate, and the back fat was sliced into 5 mm cubes. Chinese sausages were prepared according to the following formulation: lean pork (8 kg), back fat (2 kg), salt (200 g), sugar (900 g), white wine (300 g, ethanol content: 53 %, v/v, Shuanggou distillery, Jiangsu, China), sodium nitrite (1.5 g) and water (100 g). The raw materials were mixed by using a vacuum mixer machine (EXPRO Machinery Engineering Co., Ltd., Jiangsu, China). The mixture was divided into six batches of about 2 kg each. Different levels of Flavourzyme were added to the formula randomly: 0 LAPU (C), 4 LAPU (F4), 8 LAPU (F8), 12 LAPU (F12), 16 LAPU (F16) and 20 LAPU (F20) for per kg raw meat (LAPU is the unit of enzyme activity). After mixing, the mixtures were stuffed into hog casings with a diameter of 30–32 mm. Then, raw sausages (about 0.9 kg for each treatment) were linked into approximate 15 cm lengths manually and dried for 48 h at 50 °C with a pre-heated oven (KBF 115-pgm, Binder, Germany). Sausages were then taken, cooled to room temperature in 1 h, and vacuum-packaged (DC-800, Promarks Inc., USA). Immediately after the manufacture process, 8 packaged sausages of each treatment was randomly taken and kept at 4 °C, of which 4 were for texture profile analyses, and 4 were for sensory evaluation. Both texture profile analysis and sensory evaluation were carried out the next day after finished sausages were completed. The rest of the samples were then kept at −18 °C for further use. Four packaged sausages of each treatment were randomly taken for the analysis of chemical properties. The entire sausage processing procedure was replicated four times at different times.

### 2.2. Determination of moisture content, water activity (Aw) and pH

Moisture content of Chinese sausage samples was determined after dehydration (DHG-903385-III, Shanghai CIMO Medical Instrument Manufacturing Co., I.TD, Shanghai, China) at 105 °C to a constant weight by the ISO-1442 (1997) method. Water activity was detected at 25 °C using an Aw meter (LabMaster-aw, Novasina AG, Switzerland). The pH was measured according to the method of ISO-2917 (1999, IDT): 10 g sausage samples were homogenized using a blender (IKA T25 digital ULTRA TURRAX, Staufen, Germany) with 100 mL distilled water at 3000 rpm for 1 min, and the pH values were read with a pH meter (FE20 FiveEasy, Mettler Toledo, Shanghai Toledo Instrument, Co., Ltd., China).

### 2.3. Determination of protein composition

The protein components of Chinese sausage were fractionated according to the method of Sun, Cui, Zhao, Zhao, and Yang (2011) with slight modifications. Samples (5 g) were homogenized with 50 mL phosphate buffer A (15.6 mM $Na_2HPO_4$, 3.5 mM $KH_2PO_4$, pH 7.5) at 8000 rpm for 1 min in ice bath. The homogenate was centrifuged (Beckman Coulter Allegra 64R centrifuge, USA) at 5000 *g* for 15 min at 4 °C. The extraction was repeated twice. The supernatant which contained water-soluble nitrogen was combined. Half (50 mL) of the whole supernatant was kept for nitrogen determination, while the rest was combined with cold 50% TCA to a final concentration of 10 %. The resulting precipitate (water soluble protein) was gathered by centrifugation at 5000 *g* for 15 min, while the supernatant was the non-protein nitrogen (NPN) fraction. Then the pellet left by phosphate buffer A was homogenized with 50 mL phosphate buffer B (0.45 M KCl, 15.6 mM $Na_2HPO_4$, 3.5 mM $KH_2PO_4$, pH 7.5) at 8000 rpm for 1 min in ice bath and centrifuged at 5000 *g* for 15 min at 4 °C. The extraction with phosphate buffer B was repeated twice, and the supernatant was combined to obtain salt-soluble protein. The residue pellet unsolved in phosphate buffer B was collected and named as insoluble proteins. The nitrogen content of all the protein and non-protein fractions were determined by Kjeldahl method (Kjeltec™ 2300, Foss Co., Denmark). The changes in contents of every fraction along with the addition level of Flavourzyme were expressed as % total nitrogen. The water-soluble and salt-soluble proteins were analyzed by SDS-polyacrylamide gel electrophoresis according to the method of Flores et al. (2006).

### 2.4. Antioxidant capacity of peptides

#### 2.4.1. Extraction of peptides

Extraction of peptides followed the method of Sun et al. (2009) with some modifications. Samples (10 g) were added to 35 mL of 50 mM citric-sodium citrate buffer solution (pH 6.0). The mixture was homogenized using a homogenizer at 12,000 rpm for 1 min in ice bath. The homogenate was kept at 4 °C for 2 h, then centrifuged at 12,000 *g* for 15 min at 4 °C. The supernatant (water soluble peptide fraction) was filtered through qualitative filter paper (Hangzhou Whatman-Xinhua Filter Paper Co., Ltd., Jiangsu, China) and adjusted to 50 mL using buffer solution.

#### 2.4.2. DPPH radical scavenging activity (RSA)

The DPPH radical scavenging activity was measured according to the method described by Rahulan, Dhar, Nampoothiri, and Pandey (2012) and Sun et al. (2009) with some modifications. An aliquot of 100 μL of sausage extract of peptides solution was mixed with 100 μL of DPPH (0.2 mM). The mixture was kept in the dark room at room temperature for 30 min before measuring absorbance at 517 nm with a spectrophotometer (SpecturaMax M2e, Molecular Devices Corporation, USA). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (RSA). Glutathione (GSH) was used as standard, and the radical scavenging activity (RSA) of sausages was expressed as the amount of GSH in per mg sausage.

#### 2.4.3. Reducing power (RD)

The reducing power was determined according to the method described by Broncano et al. (2011). Glutathione (GSH) was used as standard, and the reducing power (RP) of sausage was expressed as the amount of GSH in per mg sausage.

### 2.5. Determination of thiobarbituric acid-reactive substances (TBARS) value

The TBARS value was measured according to the method of Wang, Pace, Dessai, Bovell-Benjamin, and Phillips (2002).

### 2.6. Texture profile analysis (TPA)

TPA was determined according to the procedure of Zhang et al. (2013) with a slight modification. Sausages were warmed to room temperature before carrying out texture analyses. Then the sausage samples were cut into 2 cm length section, and compressed twice with a Texture Analyzer (TA-XT Plus, Stable Microsystems, UK). Each