Food Chemistry 239 (2018) 556–560

Contents lists available at ScienceDirect

Food Chemistry

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

# Effect of six different starter cultures on the concentration of residual nitrite in fermented sausages during *in vitro* human digestion

Hyeong Sang Kim, Sun Jin Hur *

*Department of Animal Science and Technology, Chung-Ang University, Anseong-si 17546, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 20 March 2017
Received in revised form 7 June 2017
Accepted 29 June 2017
Available online 1 July 2017

*Keywords:*
Nitrite
Fermented sausage
*In vitro* human digestion
Enterobacteria

ABSTRACT

The objective of this study was to determine the effect of six different starter cultures of enterobacteria on the concentration of residual nitrite in fermented sausages during *in vitro* human digestion. Before digestion, the concentration of residual nitrite was dependent on starter culture in fermented sausage and ranged from 25.2 to 33.2 mg/kg. Among the six starter cultures of enterobacteria, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, and *Staphylococcus carnosus* showed higher nitrite depletion ability than the other three strains in fermented sausages. The concentration of residual nitrite in fermented sausages was significantly ($p < 0.05$) decreased after stomach digestion and ranged from 17.4 to 21.6 mg/kg. Enterobacteria *Escherichia coli* (*E. coli*) and/or *Lactobacillus casei* (*L. casei*) effectively increased the degree of depletion of residual nitrite in large intestine digestion. In conclusion, starter cultures could influence the concentration of residual nitrite during *in vitro* human digestion. They could deplete residual nitrite in fermented sausages.



## 1. Introduction

The use of nitrite in meat processing can improve the flavor, color, microbial safety, and quality of cured meats (Aksu, Erdemir, & Çakıcı, 2016; Jin, Choi, Lee, Lee, & Hur, 2016; Kang & Kim, 2016). Nitrite also can prevent deterioration by inhibiting lipid peroxidation (Karunanayaka, Jayasena, & Jo, 2016; Richards, 2013). Importantly, by inhibiting the growth of microorganisms, particularly *Clostridium botulinum*, nitrite can improve the safety of meat and meat products (Sofos, Busta, & Allen, 1979). However, nitrite in combination with certain amines or amides could potentially form *N*-nitroso compounds (NOC), which can cause cancer in animals (Honikel, 2008). Some epidemiological studies have suggested an association between dietary nitrite in red or processed meats and cancer (Abid, Cross, & Sinha, 2014; IARC, 2016), while others have demonstrated conflicting results (Bryan, Alexander, Coughlin, Milkowski, & Boffetta, 2012). Reviews and meta-analyses sometimes have different conclusions (Alexander, Weed, Cushing, & Lowe, 2011; Alexander, Weed, Miller, & Mohamed, 2015). In addition, vegetables and drinking water contribute large amounts of nitrate to the human diet, far more than cured meats (National Academy of Sciences, 1981), whereas the amounts of nitrite in vegetables and drinking water are low relative to nitrate (Bedale, Sindelar, & Milkowski, 2016). Green leafy vegetables, such as lettuce, celery, and spinach, provide large amount of nitrate, and vegetables are the dominant source of dietary nitrate in humans, contributing to 60–80% of dietary nitrate intake (Weitzberg & Lundberg, 2013). After ingestion of a nitrate-containing meal, nitrate starts its reduction to nitrite through oral commensal bacteria (Weitzberg & Lundberg, 2013). In contrast, meat and meat products usually contain residual nitrite at 0.1–12.2 ppm, which is very low compared to the content of vegetables (Nuñez De González et al., 2012).

Starter culture can influence nitrite concentration in fermented meat products. Yang, Liu, Xi, and Tang (2004) have reported that inoculation of starter cultures during vegetable fermentations is effective in lowering nitrite concentration. Yan, Xue, Tan, Zhang, and Chang (2008) have isolated lactic acid bacteria (LAB) from Chinese paocai and evaluated their nitrite depletion ability during fermentation. They reported that, among different LAB, *Lactobacillus pentosus* and *Leuconostoc mesenteroides* showed nitrite depletion ability. In addition, nitrate-reducing bacteria, such as enterobacteria, could significantly lower the formation of nitrite (Yan et al., 2008). Dodds and Collins-Thompson (1984) have also found that some *Lactobacilli* isolated from cured meat products have nitrite-reducing ability.

Although nitrite alone in food has no or limited carcinogenic potential (Grosse et al., 2006), the effects of human digestion and

* Corresponding author at: Department of Animal Science and Technology, Chung-Ang University, 4726 Seodong-daero, Daedeok-myeon, Anseong-si, Gyeonggi 17546, Republic of Korea.
*E-mail addresses:* dock-0307@hanmail.net (H.S. Kim), hursj@cau.ac.kr (S.J. Hur).

http://dx.doi.org/10.1016/j.foodchem.2017.06.160
0308-8146/

enterobacteria in human on the concentration of nitrite in fermented sausages have not been studied yet. Therefore, the objective of this study was to determine the effect of six different starter cultures on the concentration of residual nitrite in fermented sausages during *in vitro* human digestion.

## 2. Materials and methods

### 2.1. Fermented sausages

Six different kinds of fermented sausage (T1 to T6) were prepared. The sausage formulation was as follows: 84.145% pork, 9.4% pork back fat, 1.0% sea salt, 0.01% sodium nitrite, 0.045% sodium erythorbate, 0.1% sugar, 3% red pepper sauce, and 2.3% pepper mix. After trimming, chopping, and mixing the ingredients (CE93, RUHLE GMBH, Grafenhausen, Germany), the mixture was inoculated with different combinations of starter cultures. Starter culture components for the six groups of fermented sausage were: T1, *Pediococcus acidilactici*; T2, *Pediococcus pentosaceus* and *Staphylococcus carnosus*; T3, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Debaryomyces hansenii*, *Lactobacillus curbatus*, and *Pediococcus pentosaceus*; T4, *Staphylococcus carnosus* and *Lactobacillus sakei*; T5, *Staphylococcus xylosus* and *Lactobacillus plantarum*; T6, *Penicillium nalgiovensis*. Starter cultures were obtained from Almi Ges. M.g.H & Co GK (Oftering, Austria). Subsequently, the sausage mixture was stuffed into a natural casing (pig small intestine; Naturin Viscofan Co, Tajonar-Navarra, Spain) and placed in a fermentation chamber with FT 50 controller (Frigomeccanica, Parma, Italia). The first stage involved 7 days of fermentation at 4 °C. The second stage involved 30 days of fermentation at 12 °C. The relative humidity (RH) values for the first and second stage were 75–85% and 65–75%, respectively.

### 2.2. In vitro human digestion

An *in vitro* human digestion model containing mouth, stomach, small intestine, and large intestine enterobacteria was used in this study. This was a modified version of the model described previously (Lee, Lee, Chung, & Hur, 2016).

#### 2.2.1. Digestive enzymes and inorganic and organic solutions for in vitro human digestion

Digestive enzymes and inorganic and organic solutions used in this study were modified from those described previously (Hur, Lim, Decker, & McClements, 2011; Lee et al., 2016; Oomen et al., 2003; Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005). The compositions of simulated saliva, gastric, duodenal, and bile juices are listed in Table 1. After mixing all ingredients (organic and inorganic components, and enzymes, the volume was adjusted to 1 L with distilled water.

#### 2.2.2. Enterobacteria preparation used for digestion in the large intestine

Enterobacteria preparation followed the procedures described previously by Lee et al. (2016). *E. coli* was obtained from the American Type Culture Collection (ATCC) and *L. casei* MCL was isolated from feces collected from healthy adults. After small intestine digestion, enterobacteria including *E. coli* and *L. casei* were applied to samples during large intestine digestion. *E. coli* and *L. casei* were cultured to a density of log $10^9$-$10^{10}$ per mL. For the large intestine digestion system, 35 mL each of liquid-agar *E. coli* and/or *L. casei* solution were applied to samples digested in the small intestine and stirred at 37 °C for 4 h.

#### 2.2.3. In vitro digestion procedure for analysis of residual nitrite concentrations

(a) Before digestion: 5 g of fermented sausage samples were used.
(b) Mouth digestion: 5 g of fermented sausage samples were mixed with 5 mL of simulated saliva solution (pH 6.8) and then stirred in a shaking water bath (WSB-30; Daihan Scientific Co, Wonju, Korea) at 150 rpm and 37 °C for 5 min.
(c) Stomach: 10 mL of simulated gastric juice (pH 1.5) were added to samples digested in the mouth and the mixture was stirred at 37 °C for 2 h.
(d) Small intestine: 10 mL of duodenal juice and 5 mL of bile juice were added to the samples digested in the stomach and the mixture was stirred at 37 °C for 2 h.
(e) Large intestine: after digestion in the small intestine, 35 mL of liquid agar containing *E. coli* and/or *L. casei* were applied to the samples and then stirred at 37 °C for 4 h (see Section 2.2.2).

#### 2.2.4. Quantification of nitrite by high-performance liquid chromatography (HPLC)

The concentration of residual nitrite after digestion was analyzed by HPLC (HP 1100; Hewlett Packard Co., Waldbronn, Germany) on a reverse-phase column (H2o: 250 mm × 4.6 mm; 3 μm particle size; Fortis Technologies Ltd, Cheshire, UK) using water:methanol gradient (70:30, *v*/*v*) at a constant flow rate of 1 mL $min^{-1}$. The volume of sample injected for analysis was 10 μL and the detection wavelength was set at 210 nm. All samples were passed through a 0.45-μm Whatman membrane filter (Puradisc 25 NYL; Whatman, Maidstone, UK) before being injected into the HPLC column. Total run time was 15 min and retention time of nitrite was 2.193 min. Calculation of residual nitrite content of sausages samples during *in vitro* human digestion was performed using standard curves ($r$ = 0.9998). HPLC chromatogram of nitrite standard is shown in Fig. 1.

**Table 1**
Constituents and concentrations of various synthetic juices used in *in vitro* human digestion model.

| | Saliva (mouth step) | Gastric juice (stomach step) | Duodenal juice (small intestine step) | Bile juice (small intestine step) |
|---|---|---|---|---|
| Organic and inorganic components | 1.7 mL NaCl (175.3 g $L^{-1}$)<br>8 mL urea (25 g $L^{-1}$)<br>15 mg uric acid | 6.5 mL HCl (37 g $L^{-1}$)<br>18 mL $CaCl_2$ $2H_2O$ (22.2 g $L^{-1}$)<br>1 g bovine serum albumin | 6.3 mL KCl (89.6 g $L^{-1}$)<br>9 mL $CaCl_2$ $2H_2O$ (22.2 g $L^{-1}$)<br>1 g bovine serum albumin | 68.3 mL $NaHCO_3$ (84.7 g $L^{-1}$)<br>10 mL $CaCl_2$ $2H_2O$ (22.2 g $L^{-1}$)<br>1.8 g bovine serum albumin |
| Enzymes | 290 mg α-amylase<br>25 mg mucin | 2.5 g pepsin<br>3 g mucin | 9 g pancreatin<br>1.5 g lipase | 30 g bile |
| pH | 6.8 ± 0.2 | 1.50 ± 0.02 | 8.0 ± 0.2 | 7.0 ± 0.2 |