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Antioxidant and anti-foodborne bacteria activities of shiitake by-product extract in fermented sausages

Hoa Van Ba, Hyun-Woo Seo, Soo-Hyun Cho, Yoon-Seok Kim, Jin-Hyoung Kim, Jun-Sang Ham, Beom Young Park, Seong Pil Nam*

Animal Products and Processing Division, National Institute of Animal Science, 1500 kongjwipatjwi-ro, Iseo-Myeon, Wanju-gun, Jeollabuk-do, 565-851, Republic of Korea

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A B S T R A C T

The effect of addition of extract from shiitake by-products (0, 0.3, and 0.6%) on the quality characteristics, lipid oxidation and microbial stabilities of fermented sausages was studied during storage up to 30 days at 15 °C, and its effect was also compared with those produced with synthetic antioxidant, BHT (0.02%) or nitrite (0.01%). The addition of natural extract resulted in lower ultimate pH values with higher number of lactic acid bacteria, lower lipid oxidation level and spoilage bacteria count in the products during storage as compared with those of the control or BHT and nitrite treatments ($P < 0.05$). Storage time significantly increased the lipid oxidation level, however, the increasing rate after 30 days storage in the following order: Control (increased by 1.22 mg MDA/kg) > 0.02% BHT (increased by 0.43 mg MDA/kg) > 0.01% nitrite/nitrate (increased by 0.42 mg MDA/kg) > 0.3% SSE (increased by 0.34 mg MDA/kg) > 0.6% SSE (increased by 0.18 mg MDA/kg). Furthermore, extract of fermented sausages fortified with 0.6% shiitake stipes extract also showed strong antimicrobial activity against 3 foodborne bacteria such as *Staphylococcus aureus* (minimum inhibitory concentrations (MIC) = 2.08 mg/mL), *Listeria monocytogenes* (MIC = 4.16 mg/mL) and *Escherichia coli* O157 (MIC = 8.33 mg/mL). Additionally, the addition of the extract did not cause defects of color, texture and sensory quality in the products. Our results clearly suggest that the shiitake by-product extract represents a functional ingredient to be used (at level of 0.6%) for improving lipid oxidation and microbial stabilities as well as controlling the growth of pathogens in fermented sausages.

1. Introduction

Shiitake (*Lentinula edodes*), a type of edible mushroom, has long been used as part of human vegetable diet and for medical purpose in many countries worldwide especially in Asia such as; Japan, Korea and China (Jiang, Luo, & Ying, 2015; Mizuno, Sakai, & Chihara, 1995; Tian, Zhao, Huang, Zeng, & Zheng, 2016; Zhang, Cui, Cheung, & Wang, 2007). The shiitake is the second most popular edible mushroom in the global market, with total yield (based on fresh weight) produced in Asia area was approximately 4.5 million tons in 2012 (Daniel, 2014). A great amount of scientific information regarding the nutritional values of shiitake has been published in past recent years (Jiang et al., 2015; Tian et al., 2016; Zhang et al., 2007). Besides, researchers have also found that the shiitake contains high levels of bioactive compounds such as phenols and polyphenols etc. that exhibit a wide range of biological properties such as antimicrobial, antioxidant, anticancer, antidiabetic and antihypertensive effects (Choi, Lee, Chun, Lee, & Lee, 2006; Reis, Martins, Barros, & Ferreira, 2012; Zhang et al., 2010). Regarding the antibacterial effect, the shiitake has been proven to have activity against some pathogenic bacteria such as *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus megaterium* etc. (Hatvani, 2001; Kitzberger, Artur, & Pedrosa, 2007).

Structurally, the shiitake consists of two parts that are cap and stipe, in which the cap is used whereas the stipe is usually discarded due to its tough texture (Zhang, Chen, Zhang, Ma, & Xu, 2013). However, researchers have proven that the stipe part has higher not only nutritional value but also levels of bioactive compounds than the cap part (Yen, Tseng, Li, & Mau, 2007; Zhang

* Corresponding author.
E-mail address: hoavanba@jbnu.ac.kr (H. Van Ba).

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et al., 2013). Additionally, the consumption of shiitake has increased greatly in recent times (Reis et al., 2012), meaning that a considerable amount of their stipes considered as waste, is produced every day from mushroom farms and processors, which becomes a burden problem in disposing of them when they are not utilized. However, this abundant available resource may also produce good opportunities for the food industry to increase economic profitability if these mushroom by-products are processed and utilized in a suitable way. Therefore, investigations to generate new value-added products from these discarded shiitake stipes are needed.

Practically, fermented sausages are produced from raw materials, commonly about 70–80% meat and 20–30% pork fat, and together with additives, spices, starter culture and nitrite curing salt (Nassua, Goncalves, da Silva, & Beserra, 2003; Kurćubić et al., 2014). Due to the high added level of fat and typical processing technology (e.g., using raw materials without pasteurization and no heat treatment is applied) the product is therefore highly susceptible to the oxidative agents, and favoring the growth of undesirable bacteria (spoilage and pathogenic bacteria), which reduces shelf-life stability of the product and has an implicit hazard to consumers as well. On the other hand, despite the fermentation process of raw materials results in low pH and water activity which basically inhibit the growth of most pathogenic bacteria (Leistner, 2000), however, researchers have indicated that some pathogens such as *Listeria monocytogenes* and *Escherichia coli* can survive in fermented sausages and may not be completely eliminated (Lindqvist & Lindblad, 2009). In recent years, a large number of foodborne outbreaks of *E. coli* (verotoxin-producing *E. coli* with different serotypes such as O157, O103, O26 and O111 etc.) caused by raw cured sausages have been reported in many countries (Holck et al., 2011). Typically, the recently occurred outbreak in Norway in 2006 resulted in food poisoned 18 cases (one of them died) (Sekse et al., 2009). The control of undesirable bacteria growth therefore is necessary for the shelf-life extension and microbiological safety of the fermented sausages.

Nitrite and nitrate are commonly used as curing agents and preservatives in fermented sausages production due to their antioxidant and antimicrobial effects (Cammack et al., 1999). Despite the nitrite is important in term of technology, however, it's association with potential health risks leads to the formation of methemoglobin and carninogenic N-nitrosamines which are potentially toxic compounds in human (Corpet, 2011). The use of nitrate in the food industry is therefore strictly regulated. Otherwise, the use of available synthetic antioxidants (e.g., Butylated hydroxytoluene, BHT) has been suspected to cause toxicity problems that negatively affect consumer's health (Botterweck, Verhagen, Goldbohm, Gleinjans, & Brandt, 2000). Therefore, a new trend to substitute these synthetic antioxidants with antioxidants from natural sources (e.g., plant and herb extracts) have been received the most attention by consumers and meat processors (Chaves-López et al., 2015; Kurćubić et al., 2014; Lorenzo, González-Rodríguez, Sánchez, Amado, & Franco, 2013; Seong et al., 2015) because the use of natural antioxidants and antimicrobials has the advantage that they are readily accepted by the consumer (Pokorny, 1991).

While the shiitake stipes are an abundant material which contains high levels of bioactive compounds with potent antioxidant activity as proven in previous studies, however, no attention has been paid to its processing and utilization as natural preservatives to substitute for the synthetic preservatives in food products in general and meat products in particular. The objective of the present study was to assess the impacts of incorporation of shiitake stipes extract on the quality characteristics and shelf-life stability (antioxidant and antimicrobial) of fermented sausage.

2. Materials and method

2.1. Materials

Shiitake stipes were collected from a local commercial mushroom production farm in Jeonju, South Korea. Fresh pork meat and back-fat were obtained from a local commercial processor (Jeonju, Korea) 24 h after slaughter. The chemicals used in the present study including: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Butylated hydroxytoluene (BHT), 1,1,3,3 tetramethoxypropane, gallic acid, resazurin indicator solution, Folin–Denis's reagent, Mueller Hinton broth and ciprofloxacin were purchased from Sigma Chemical, CO. (St. Louis, MO, USA). Tryptic soy broth and tryptic soy agar were purchased from Becton, Dickinson and Company (Sparks, MD, USA). The rest of ingredients such as sodium nitrite and sodium nitrate (Shinyo Pure Chemicals, Co. Osaka, Japan), sodium chloride (Hanju Co., Ulsan, Korea) and starter culture (Almi. 2, Almi Ges. m.b.H & Co GK, Oftering, Austria) were used for fermented sausages production.

Standard cultures of food pathogens: *Escherichia coli* O157:H7 ATCC 43894, *Staphylococcus aureus ATCC 25923, Listeria monocytogenes ATCC 15313* and *Salmonella typhimurium* KCCM (Korean Culture Center of Microorganisms) 11,862 were used for antimicrobial activity test.

2.2. Preparation of shiitake stipes extracts (SSE)

The shiitake stipes were washed with water to remove any impurities, and then were sliced into thin slices and dried by freezing-dry at −50 °C. The dried stipeses were powdered using a blender and then were used for extraction. The SSE was obtained using the method of Zhang et al. (2013) with suitable modifications. Briefly, the powdered stipes (50 g, each extraction batch) was extracted by stirring with 300 mL of distilled water at 100 °C on a shaker for 2 h and filtered through Whatman filter paper. The filtrates were dried at 50 °C, and the dried SSE was then stored at 4 °C to prevent oxidative damage until use.

2.3. Formulation and processing of fermented sausage (FS)

In the present study, five formulations of fermented sausage treatments were prepared. Each treatment had 3 batches and each batch was prepared with 7 kg of meat batter. All batches were made with 70% pork (*semimembraneous, semitendinous adduct, biceps femoris, quadriceps femoris, middle gluteal* and *gracilis* muscles) and 30% pork back-fat, and together with the following additives: NaCl 20 g/kg, sugar 15 g/kg and starter culture (*Staphylococcus carnosus*, *Staphylococcus xylosus, Debaryomyces hansenii* and *Lactobacillus curvatus*) 0.2 g/kg. The particular treatments (T) were prepared as follows: T1 and T2 were added with 3 and 6 g/kg of the SSE, respectively (the SSE was prepared into solution by dissolving the defined amounts in 100 mL of distilled water before adding to the corresponding treatments, and the initial pH value of SSE solution was 5.62). T3 was added with 0.01% (w/w) $NaNO_2$ and 0.005% (w/w) $NaNO_3$, while T4 added with 0.02% (w/w) (BHT) was served the positive control. The batches made with the meat mixture and additives without SSE were served as the negative control (Fig. 1). To determine whether the SSE exerts its antioxidant and antimicrobial activities in fermented sausage, the common seasonings such as garlic and pepper etc. were not added because these spices may also possess some antioxidant and antimicrobial characteristics which may interfere and mask the real effect of the added SSE content in the product.

The trimmed meat and fat were chopped through a 3 mm plate using a silent chopper (Model 7548, Biro MFG. Co, Ohio, USA). For