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Effects of extraction methods of shiitake by-products on their antioxidant and antimicrobial activities in fermented sausages during storage

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ABSTRACT

Two different solvents (water and ethanol/water at 50/50 ratio) were used for extraction of phenolic contents in shiitake by-products, and the antioxidant and antimicrobial efficacies of fermented sausages incorporated with these two extracts were investigated during storage. Also, shelf-life stability of fermented sausages fortified with these two extracts was compared with those fortified with BHT (0.02%) or nitrite (0.01%)/nitrate (0.005%). Significantly higher antioxidant activities (e.g., ferrous ion chelating and DPPH scavenging) and inhibitory capacity against lipid oxidation (e.g., TBARS reduction) were observed in the fermented sausages made with ethanolic extract (shiitake by-products extracted with ethanol/water) compared with those added with aqueous extract (extracted with water) or BHT, nitrite/nitrate and control throughout storage. The ethanolic extract treated-samples also showed a significantly slower increasing rate of total aerobic count (6.54–6.95 $\log_{10}$ cfu/g) than the ones treated with aqueous extract (6.74–7.16 $\log_{10}$ cfu/g) during storage. Otherwise, extract obtained from the ethanolic extract treated-samples had stronger antimicrobial activities against pathogens than the one obtained from aqueous extract treated-samples (e.g., minimum inhibitory concentrations, MIC = 2.05 & 3.64, 3.12 & 5.20 and 7.29 & 10.41 mg/mL for *S. aureus, L. monocytogenes* and *E. coli* O157, respectively). Especially, the antimicrobial activity against *S. typhimurium* (MIC = 37.50 mg/mL) was observed only in the extract of ethanolic extract treated-samples. Our study demonstrates that the extraction with ethanol/water solvent is a more effective method to obtain bioactive compounds enriched-extract which better improved the shelf-life stability of fermented sausages during storage without defects in quality in comparison to the extraction method with water.

1. Introduction

Fermented sausages are uncooked meat products that have high risk of contaminations with spoilage and pathogenic bacteria, and high lipid oxidation level, which reduces shelf-life stability of the product and has an implicit hazard to consumers (Ferreira et al., 2007; Heir et al., 2013; Kuhn, Torpdahl, Frank, Sigsgaard, & Ethelberg, 2011; Sekse et al., 2009). Studies have reported that some pathogens (e.g., *Listeria monocytogenes, Salmonella* and *Escherichia coli* etc.) can survive in fermented sausages (Ducic et al., 2016; Lindqvist & Lindblad, 2009) and they have caused a large number of foodborne disease outbreaks in many countries (Heir et al., 2013; Holck et al., 2011; Sekse et al., 2009). Furthermore, the spoilage of meat products during storage resulting in discoloration, undeniable appearance and off-flavors (Cerveny, Meyer, & Hall, 2009), is mainly caused by the activity of bacterial spoilers such as *Enterobacteriaceae* and especially Gram-negative, aerobic bacteria (Garcia-Lopez, Prieto, & Otero, 1998). The control of foodborne and spoilage bacteria growth therefore is needed in order to ensure the microbiological safety of the uncooked meat products like fermented sausages. On the other hand, although nitrite and nitrate are the most commonly used as preservatives in fermented sausages production due to their antioxidant and

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antimicrobial effects (Cammack et al., 1999), however, the use of these salts has proven to have potential health risks due to the formation of toxic compounds such as carninogenic N-nitrosamines (Corpet, 2011). Additionally, the use of synthetic antioxidants such as Butylated hydroxytoluene (BHT) has been found to cause toxicity problems (Botterweck, Verhagen, Goldbohm, Gleinjans, & Brandt, 2000). Therefore, the substitution of synthetic compounds by antioxidants/antimicrobials from natural sources 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).

Among plant chemicals, phenolic contents possess a great scientific interest; these compounds are produced by plants as antioxidant and protective substances against environmental stresses (Pandey & Rizvi, 2009). From a health benefit point of view, the phenolics have been proven to exert beneficial effects on human health due to their activities against cancer, cardiovascular diseases and allergic and ulcerous disorders etc. (Shahidi & Ambigaipalan, 2015).

Shiitake *(Lentinula edodes)* is the second most popular edible mushroom in the global market (Bisen, Baghel, Sanodiya, Thakur, & Prasad, 2010; Daniel, 2014). The mushroom has long been used as vegetable diet and for pharmaceutical use in many countries worldwide (Jiang, Luo, & Ying, 2015). Past recent years, a large number of studies have shown that the mushroom is rich in nutrients and bioactive compounds as well as their biological properties (Jiang et al., 2015; Reis, Martins, Barros, & Ferreira, 2012; Zhang et al., 2010). Regarding the structure, shiitake consists of two separate parts that are cap (the main body) 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, the stipe part has been proven to contain higher not only nutritional value but also levels of bioactive compounds (e.g., phenolics) than the body part (Yen & Mau, 2007; Yen, Tseng, Li, & Mau, 2007; Zhang et al., 2013). Additionally, due to the increasingly consumed amount of shiitake in recent times (Reis et al., 2012), a significant amount of their stipes (by-products) considered as waste materials, is produced every day from mushroom farms and processors. However, this abundant available resource may also produce possibilities and good opportunities for the food industry to increase economic profitability if these mushroom by-products are processed and utilized in a proper way. Therefore, investigations to produce new value-added products from these discarded by-products are needed.

Furthermore, the results of our previous examinations on antioxidant and antimicrobial activities of shiitake stipes extract in fermented sausages (Ba et al., 2016a) were the basis to continue with the current research on finding out a more effective extraction method with high phenolic content yield which could better improve the oxidative stability and microbiological quality of the products. It has been found that different solvents or extraction methods significantly affect the yield of phenolic contents in plants (Do et al., 2014; Masci et al., 2016; Onivogui, Letsididi, Diaby, Wang, & Song, 2016). Moreover, bioactive compounds with different chemical and polarity characteristics may or may not be soluble in a particular solvent (Turkmen, Sari, & Velioglu, 2006). So far, methanol, ethanol and acetone etc. are the most commonly used polar solvents for the extraction of polyphenols in plants. Comparing to methanol or others, ethanol has been known as a good solvent for polyphenols extraction and is considered safer for human consumption (Akhtar, Ismail, Fraternale, & Sestili, 2015; Turkmen et al., 2006). Recent reports have shown that the use of 50% ethanol as an extractant allowed obtaining higher polyphenolic contents from plant sources compared to higher ethanol level (e.g., 80–100%) or water alone use (Do et al., 2014; Turkmen et al., 2006).

Therefore, we hypothesized that different solvent types may affect the quantity and quality of phenolic contents in shiitake stipes, which may subsequently affect their bioactivities after being added into meat products. Moreover, the application of each plant extract in food industry requires the efficacy evaluation within real food products and should be supported by sensory quality evaluation. Thus, the present work aimed at comparing the antioxidant and antimicrobial efficacies between two shiitake stipes extract types (ethanolic and aqueous extract) in fermented sausages during storage. Our final goal was to find out a more effective extraction method of phenolic contents in shiitake stipes in order to obtain the phenolic contents enriched-extracts which can prolong the shelf-life stability and better improve microbiological safety of fermented sausages without defects in the qualities of the products.

2. Materials and methods

2.1. Materials

Shiitake stipes and all the chemicals used were the same as those described in previous study (Ba et al., 2016a). Starter culture (Almi. 7) was purchased from Almi Ges. m.b.H & Co GK (Oftering, Austria). Standard cultures of pathogenic bacteria: *Escherichia coli* O157:H7 ATCC 43894, *Staphylococcus aureus ATCC 25923, Listeria monocytogenes ATCC 15313* and *Salmonella typhimurium* KCCM (Korean Culture Center of Microorganisms) 11862 were used for antimicrobial activity test.

2.2. Preparation of shiitake stipes extract types

The shiitake stipes collected from a local commercial mushroom production farm (Jeonju, South Korea) were washed with water to remove any impurities, sliced into thin slices and then dried by freezing-dry at −50 °C. The dried shiitake stipes were powdered using a blender and then used for extraction. In order to find out a more effective extraction method which allows obtaining higher phenolic contents in shiitake by-products and without negative effect on the quality of fermented sausages, two different extraction methods including: (i) extraction with distilled water (100%), so called “aqueous extract” and (ii) extraction with a mixture of ethanol/water (50:50 ratio), so called “ethanolic extract” were used in the present work. Based on the results obtained in our previous work (Ba et al., 2016a) in that the addition of 0.6% (w/w) of shiitake stipes extract (extracted with water alone) showed the effectiveness in improvement of shelf-life stability of fermented sausages during storage up to 30 days. Therefore, in the present study, the same amounts of shiitake stipes powder (the weight of shiitake stipes powder needed to produce the 0.6% extract) were used for each extraction method. The aqueous and ethanolic extracts were obtained using the procedures as described by Zhang et al. (2013) and Lorenzo et al. (2013), respectively with suitable modification. The extraction procedures such as volumes of solvents and conditions used were same for the both methods. Particularly, the shiitake stipes powder (100 g, each extraction batch) was extracted by stirring with 500 mL of distilled water alone or ethanol/water mixture at 100 °C on a shaker at 300 × rpm for 2 h and filtered through Whatman filter paper. The filtrates were dried at 50 °C, and the dried extracts were then stored at 4 °C to prevent oxidative damage until use.

2.3. Formulations 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 about 7 kg of meat batter. All treatment