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Effect of *Flammulina velutipes* on the physicochemical and sensory characteristics of Cantonese sausages

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Abstract

The effects of fresh and dried *Flammulina velutipes* (FFV and DFV) on quality and sensory characteristics of Cantonese sausages were investigated. Sausage samples were prepared by adding 0% (control), 2.5% FFV, 2.5% and 5.0% DFV, respectively, and their nutritional compositions, free amino acid profiles, lipid and protein oxidation, color and texture properties were determined. Addition of *F. velutipes* significantly decreased fat content while increased free amino acid contents of Cantonese sausages. Total free amino acid contents of 2.5% FFV, 2.5% DFV and 5.0% DFV incorporation were 2.8-, 2.4- and 3.5-fold as compared to control, respectively. Lipid and protein oxidation of Cantonese sausages were effectively inhibited by the addition of *F. velutipes*. Both FFV and DFV addition decreased hardness and chewiness while showed different effect on yellowness of samples. DFV added at 2.5% exhibited the best overall sensory acceptance. Therefore, appropriate addition of *F. velutipes* may be an effective way to improve meat product quality and function.

Keywords: *Flammulina velutipes*; Cantonese sausage; Protein oxidation; Lipid oxidation; Sensory properties

1. Introduction

Meat and meat products have been widely acknowledged as important sources for essential nutrients including protein, fatty acids, vitamins, minerals and other bioactive compounds (Olmedilla-Alonso, Jiménez-Colmenero, & Sánchez-Muniz, 2013). However, they also contain large amount of lipids, cholesterol, and saturated fatty acids which, when consumed in inappropriate quantities, may increase the risk of some degenerative and chronic diseases (WCRF/AICR, 2007; WHO/FAO, 2003). Therefore, there is a marked interest in designing and developing meat-based functional products by incorporating other food ingredients containing health promoting bioactive compounds (Olmedilla-Alonso, Jiménez-Colmenero, & Sánchez-Muniz, 2013).

Cantonese sausage, a Chinese traditional semi-dry cured meat product (mainly in Guangdong Province), has long been popular with consumers. However, its market size tends to shrink in recent years due to its limited product range and high contents of sugar and salt as well as nitrite. Hence, it is essential to develop functional formulation for this traditional meat product to address consumer needs and to improve its nutritional and dietary properties. Although many different strategies have been reported to introduce bioactive compounds (endogenous and exogenous) in meat and meat products, incorporating functional components from healthy plants to meat products is one of the most important approaches in the development of meat-based functional foods (López-López, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2009). Therefore, numerous raw ingredients (mainly from plants and fungi) have been used to reformulate meat products so as to promote the presence of healthy compounds (Rosli, Maihiza, & Raushan, 2015; Van Hecke, Ho, Goethals, & De Smet, 2017).

Flammulina velutipes, also known as golden needle mushroom, winter mushroom and enokitake, has been artificially cultivated in large-scale in Asian countries, especially in Japan and China (Cai, Liu, Chen, Liao, & Zou, 2013). *F. velutipes* is rich in fiber, protein, amino acids, and minerals but low in fat (Yi et al., 2013). In addition, this mushroom contains quite a few of biological active compounds including large molecules (bioactive protein, polypeptide, polysaccharide, β-glucan, heterogalactan, mannofucogalactan, etc.) and small ones (sterols, ergothioneine, cuparene sesquiterpenes, dilinolein, hemisceramide, etc.) (Cai, Liu, Chen, Liao, & Zou, 2013). Furthermore, *F. velutipes* has been correlated with multiple pharmacological activities such as antioxidant, immunomodulatory, cholesterol-lowering, hypoglycemic and anti-cancer activities (Dong, Cheng, Qi, Yang, Yin, & Chen, 2017; Leung, Fung, & Choy, 1997; Wu et al., 2014). Thus, *F. velutipes* may be a valuable natural ingredient used to design meat-based functional foods (Ioannis, 2014). Recently, researches indicated that *F. velutipes* could be used as an alternative to phosphates in emulsion-type sausages (Choe, Lee, Jo, Jo, Song, & Jung, 2018) and as a tenderizer to improve the texture of spent-hen meat (Kang et al., 2012). Usually, mushroom contains large amounts of ergosterol, fungisterol and endogenous enzyme. Herein, we hypothesized that *F. velutipes* could be used as a food ingredient to improve the quality of Cantonese sausages, and that different treatment of *F. velutipes* might result in different effects.

The aim of the present work was to evaluate the effects of fresh and dried *F. velutipes* on the nutritional composition, free amino acid profile, lipid and protein oxidation, color and texture properties and sensory characteristics of Cantonese sausages. This work is performed in order to provide alternatives for improving the quality and nutrition properties of Cantonese sausages, and enhance its customers' accepatance.

2. Materials and methods

2.1. Materials and chemicals

F. velutipes were purchased from a local market in Guangzhou, China. Fresh *F. velutipes* (FFV) were washed with tap water, drained and then minced by a bowl cutter (SZ-12A, Guangzhou Xuzhong Food Machinery Co., LTD, China). The dried *F. velutipes* (DFV) was prepared by drying the washed *F. velutipes* using a GHRH-20 heat pump (Guangdong Agricultural Machinery Institute, Guangzhou, China) at 60 ℃ for 36 h, and then grounded into powder by a BJ-300 high speed multifunction agitator (Baijie Company, Hangzhou, China). The water contents of FFV and DFV were determined, and the FFV and DFV added into the sausages were calculated as dry matter of *F. velutipes*. Lean pork and back pork fat were purchased from local markets in Guangzhou, China. All other chemicals and reagents were purchased from Qiyun Company (Guangzhou, China) and Sigma-Aldrich.

2.2. Sausage preparation

Cantonese sausages were prepared according to Wang, Zhou, Cheng, Chen, & Liu (2018) with slight modifications. The lean pork was minced and the back fat was cut into approximately 5 mm cubes. Lean pork (800 g) and back fat (200 g), salt (23 g), sugar (120 g), Chinese liquor (20 g), sodium nitrite (0.2 g) and MSG (2 g) were mixed with a silent cutter (CH-10, Zhongnan Pharmaceutical Machinery Factory, Changsha, Hunan, China). The 2.5% FFV, 2.5% DFV and 5.0% DFV were added based on meat weight. Different level of FFV and DFV were added depending on the formula for each of the four treatments: 1) control: sausage manufactured without FFV or DFV addition; 2) 2.5% FFV: sausages manufactured with 2.5% FFV addition; 3) 2.5% DFV:

sausages manufactured with 2.5% DFV addition; 4) 5.0% DFV: sausages manufactured with 5.0% DFV addition (Table 1). As fresh *F. velutipes* contained large amount of water, if 5.0% was added it would introduce too much water into the sausage and severely affect the appearance of the product. Therefore, we only tested the effect of 2.5% addition for fresh mushroom. After thorough mixing the meat and incorporated ingredients, the mixtures were filled into cellulose casings (40 mm diameter) using a sausage stuffer, ligated with straw rope into approximately 15 cm lengths and oven-dried for 8 h at 45 °C, followed by 50 °C for 40 h. At the end of the drying, one part used for sensory analysis, and the others were stored for quality analysis. Samples for analysis of nutritional composition, free amino acids, instrumental color and texture properties were stored at 4℃for one to two days. Samples for lipid and protein oxidation analysis were stored at -80 ℃until measurements.

2.3. Nutritional analysis

Moisture, fat (Soxhlet method) and ash contents were determined according to AOAC (2005). Protein was measured by Kjedahl method with a nitrogen detector (Kjeltec™ 8400 Analyzer Unit, Foss Analytical AB, Sweden, China).

2.4. Free amino acid analysis

Free amino acids were extracted from sausages and quantified according to a method previously reported by Chen, Kong, Han, Liu, & Xu (2016) using a Hitachi L-8800 (Hitachi Co., Ltd., Japan) equipped with UV–visible detector and #2622PF column (4.6 mm × 60 mm). Free amino acid values were expressed as mg/100 g sausage.

2.5. Lipid oxidation

Peroxide value of sausage was determined according to the method of Kaewprachu, Osako, Benjakul, Suthiluk, & Rawdkuen (2017) with some modifications. Lipid was extracted from sausage as follows: A 20 g minced sausage mixed with petroleum ether in proportion of 1:3 (w/v), after shaking vigorously, the mixture was then kept at room temperature for 12 h. Subsequently, the mixture was filtered through anhydrous sodium sulfate, and the filtrate was collected. After removing the solvent by rotary evaporation, the residue was the extracted lipid inform the sausage. The extracted lipid (2 g) was dissolved in 30-mL mixtures of chloroform/acetic acid (1:2), treated with 1 mL of saturated potassium iodide solution and kept in the dark for 5 min. Then one hundred milliliters of distilled water was added to the mixture and shaken vigorously; one milliliter of fresh starch solution (1.0% w/v) was added as an indicator. The lipid peroxidation was determined by titrating iodine liberated by the potassium iodide with sodium thiosulphate solution. The peroxide value was defined as the oxidized potassium iodide content, expressed as g/100 g of lipid.

Thiobarbituric acid-reactive substances (TBARS) value was measured according to the method of Cheng et al., (2017). TBARS value was expressed as mg of malondialdehyde (MDA) per 100 g of sausage using tetraethoxypropane as a standard.

2.6. Protein oxidation

Protein carbonyls and sulfhydryl contents were determined according to the method of our previous report (Cheng et al., 2017). Protein concentrations were calculated by measuring the absorbance at 280 nm, using BSA as standard. The contents of carbonyl and sulfhydryl groups were expressed as nmol/mg of protein.

2.7. Color evaluation

A chromometer (UItraScan VIS, Chroma Meter, US) was used for color measurement of sausages. The colorimeter was calibrated with a white tile (L*=93.16, a*=−0.79, b*=1.28) prior to color measurement. Sausage sample was minced by a BJ-300 high speed multifunction agitator (Baijie Company, Hangzhou, China), then the minced sausage was put into a transmission cell (5 mm × 5 mm × 1 mm) and fixed on the reflectance port by a swing sample clamp. Color measurements, L* value (lightness), a* value (redness) and b* value (yellowness), were made on the surface of the minced sausage in triplicate at three randomly selected locations, at room temperature (25 °C) with illuminant D_{65} and a 10° angle.

2.8. Texture analysis

Texture analysis was performed by using a texture analyser (TA-XT plus, Stable Micro Systems, Surrey, England) fitted with a cylindrical probe (P/50, 50 mm stainless cylinder) as described by Choe et al., (2018) with some modifications. Sausage samples were cut into 25 mm height cylindrical shape. The settings were fixed at a pre-test speed 1.0 mm/s, test speed of 5.0 mm/s, and post-test speed of 5.0 mm/s. For probe height calibration, 30 mm distance, 10 mm/s return speed and 5 g contact force were used. The samples were then placed on the center of the platform and the analysis was run at 50% strain. The texture characteristics of sausage were expressed as the hardness, springiness, cohesiveness and chewiness.

2.9. Sensory evaluation

Twenty panel members were selected and trained according to Lawless & Heymann (2010) from students and staffs who are familiar with the products. Training sessions were conducted according to the criteria described by Carpenter, Lyon & Hasdell (2010). The sensory properties of sausages from 4 treatments were evaluated twice in two independent sessions over two days. Samples were cooked with water steam for 8 min, then cut into 1 cm sections, placed on plates and served to the panelists. The sample order was randomized within the sessions. A glass of about 100 mL of water was provided to gargle for each panelist between samples. Each individual panelist was asked to evaluate the sensory attributes of sausage samples, including color, texture, odor, taste and overall-acceptability. A 9-point hedonic scale (1 = strongly dislike, 9 = strongly like) was used to score the samples.

2.10. Statistical analysis

The entire experimental design was followed in triplicate and difference between replicates are not significant ($p<0.05$). The effect of different *F. velutipes* treatments on nutrition, free amino acids, lipid and protein oxidation, color, texture and sensory parameters were examined using a mixed-model ANOVA, where different *F. velutipes* treatment as fixed effect, and replicate as random effect. The pairwise differences between least-square means were evaluated by Duncan's method. Difference were considered significant if $p<0.05$. The values were given in terms of mean values and standard error. All statistical analyses were performed using SPSS Statistics 17.0 software (SPSS Inc., Chicago, Illinois).

3. Results and discussion

3.1. Effects of *F. velutipes* on nutritional compositions of Cantonese sausages

The nutritional compositions of Cantonese sausages formulated with different concentrations of FFV and DFV are shown in Table 2. The moisture contents in all Cantonese sausages range from 18.48% to 19.87%. No significant differences ($P>0.05$) were observed among the addition of

2.5% FFV, 2.5% DFV and control groups. Although significant difference ($P<0.05$) in moisture content between control and 5.0% DFV addition was observed, the statistical difference was only quantitatively 1% which could be acceptable in the three batch performance.

Cantonese sausage supplemented with 2.5% FFV recorded the highest protein content (28.3%) among all tested groups, while no significant difference was observed as compared to control samples. It may be due to the dry matter of *F. velutipes* mushroom contained slightly higher or similar amount of protein compared to meat. As showed in Table 2, the protein content of *F. velutipes* was 22% on a dry matter basis. However, the protein content of meat was about 20% to 22% (Sánchez-Macías, Barba-Maggi, Morales-delaNuez, & Palmay-Paredes, 2018; Seiquer et al., 2019). As expected, no significant difference in protein content was observed among 2.5% DFV, 5.0% DFV and control samples. It is noteworthy that the protein content in Cantonese sausage containing 2.5 % FFV was higher than those in DFV groups with 2.5% (26.4%) and 5.0% (26.7%) addition.

Fat content was inversely proportional to the percentage of *F. velutipes* added to Cantonese sausages. Control had the highest fat content (28.9%), followed by 2.5% FFV (26.2%), 2.5% DFV (24.7%) and 5.0% DFV (22.1%) group. This result was similar to that reported in an earlier study on chicken frankfurters with partially replacement of meat by *Pleurotus sajor-caju* (Rosli, Maihiza, & Raushan, 2015). But, there was no significant difference ($P>0.05$) in fat content between 2.5% FFV and 2.5% DFV treatments, due to the low fat content in *F. velutipes* (Kang, Zeng, Ye, Lin, & Guo, 2014). It is well known that low fat content is a common feature for edible mushrooms (Cheung, 2010; Kalač, 2009).

Ash content of the Cantonese sausages ranged from 6.12% to 6.32% was observed as shown in Table 2. Incorporation of *F. velutipes* did not affect ash contents of Cantonese sausages. Similar pattern was also reported in previous studies on sausages supplemented with inulin, oat fiber and wheat fiber (Mendoza, García, Casas, & Selgas, 2001; Rosli, Maihiza, & Raushan, 2015).

3.2. Effects of *F. velutipes* on free amino acids of Cantonese sausages

The degradation of amino acids have been associated with the ripened aroma of cured meat products (Hospital et al., 2015). The effects of FFV and DFV on free amino acid contents of Cantonese sausages are summarized in Table 3. In the present study, 25 free amino acids were detected in the end products and the order of total free amino acid content was 5% DFV (2262.29 mg/100 g), 2.5% FFV (1864.63 mg/100 g), 2.5% DFV (1575.18 mg/100 g) and control (651.70 mg/100 g). In general, Glu, Tau, Ala, Lys, Leu and Arg were the most abundant amino acids in the sausage samples. These results were similar to those found in European dry-fermented sausages (Gallego, Mora, Escudero, & Toldrá, 2018). Interestingly, total free amino acid contents in all three groups added with *F. velutipes* were not only remarkably higher than the control, but also significantly higher than the sum of free amino acids in control and in the added *F. velutipes* alone. This is due to proteases present in edible mushroom (Grimrath, Berends, Rabe, Berger, & Linke, 2011; Lee et al., 2017; Shin et al., 2008). Those protease/peptidase in the mushroom degrade meat protein into free amino acids, and *F. velutipes* has been widely used for the tenderization of meat due to its hydrolysis activity (Kang et al., 2012).

As compared to control samples, the contents of free amino acids, except Tau, were significantly higher in the Cantonese sausages incorporated with FFV and DFV. On one hand, these results are due to the abundant free amino acids in *F. velutipes,* except for Tau which was not detected in this mushroom. On the other hand, proteolytic enzymes in the mushroom played important roles in the degradation of meat protein in the sausage. As expected, the addition of 5.0% DFV to the Cantonese sausages gave rise to a significantly higher amount of free amino acids than that of 2.5% DFV. However, no significant difference was observed on some free amino acids between sausages incorporated with 2.5% FFV and 2.5% DFV or 5.0% DFV. These results may be explained by the fact that the drying process affected the amount of free amino acid in *F. velutipes*. The present results indicate that the amount of free amino acids in 2.5% FFV was more than 2.5% DFV, while less than 5.0% DFV. In addition, the contents of Glu, Ala, Lys, α-ABA, Orn, His and Arg in FFV were higher than those in DFV. Similarly, it was reported that fresh shiitake mushrooms had significantly higher amounts of Ala than the dried samples (Tian, Zhao, Huang, Zeng, & Zheng, 2016). Although partial free amino acid contents of *F. velutipes* increased after drying processing, the total free amino acid content decreased in the dried samples. Therefore, the addition of FFV in Cantonese sausages gave rise to a significantly higher amount of free amino acids at the end of ripening.

3.3. Effects of *F. velutipes* on lipid oxidation of Cantonese sausages

The peroxide and TBARS values are the two main indicators of lipid oxidation of meat products. The effects of FFV and DFV addition on peroxide and TBARS values of Cantonese sausages were presented in Figure 1. Cantonese sausages with the addition of FFV and DFV had much lower peroxide and TBARS values than the control ($P < 0.05$). This result may be attributed to *F. velutipes* contained ergothioneine (Pahila, Kaneda, Nagasaka, Koyama, & Ohshima, 2017), polysaccharide (Wu et al., 2014), phenolic compounds and flavonoids, which are well-known antioxidants (Jiang & Xiong, 2016).

As clearly seen in Figure 1A, significant differences ($P <0.05$) in peroxide appeared among the three *F. velutipes* addition groups and control. The highest peroxide value was found in control (0.10 g/100 g lipid), while the lowest was observed in 2.5% FFV group (0.02 g/100 g lipid). Similarly, as shown in Figure 1B, the highest TBARS value was determined in control samples (3.4 mg/100 g sausage). However, no significant differences (P>0.05) in TBARS value were observed among the three *F. velutipes* addition groups. The lowest TBARS value was observed in samples containing 5.0% DFV (1.9 mg/100 g sausage), which decreased by approximately 47% compared with control. The greater DFV level of addition, the lower TBARS value was detected; and the TBARS value of 2.5% FFV addition was lower than that of 2.5% DFV addition. It seems that FFV is superior to DFV in protecting lipid against oxidation. This might be ascribed to the drying process damaging bioactive compounds, as they are sensitive to heat and oxygen (de Torres, Díaz-Maroto, Hermosín-Gutiérrez, & Pérez-Coello, 2010; Demirkol & Tarakci, 2018).

3.4. Effects of *F. velutipes* on protein oxidation of Cantonese sausages

It has been reported that protein oxidation has negative effects on meat quality including texture, flavor, water-holding capacity, and biological functionality (Cheng et al., 2017). In the present study, the formation of protein carbonyls as well as the loss of sulfhydryl content was determined to evaluate the effects of FFV and DFV on the protein oxidative deterioration in Cantonese sausages (Figure 2).

Cantonese sausages supplemented with FFV and DFV showed significantly lower protein carbonyl contents than in control (P < 0.05) (Figure 2A). The more DFV added, the lower the formation of protein carbonyls measured. Samples with addition of 2.5% FFV showed the lowest carbonyl content. Compared with control, carbonyl contents in sausages with 2.5% FFV, 2.5% DFV and 5.0% DFV addition decreased 50%, 22% and 40%, respectively. These results indicated that the higher the amount of plant antioxidant used, the lower the protein oxidation occurring (Turgut, Soyer, & Işıkçı, 2016).

The total sulfhydryl contents of Cantonese sausages followed a different pattern which was in line with Cheng et al. (2017), who reported that *Momordica grosvenori* extract protected protein against oxidation in dried minced pork slices. The effects of FFV and DFV on sulfhydryl contents of Cantonese sausages are presented in Figure 2B. Compared with control, the sulfhydryl contents significantly increased from 64.34 nmol/mg to 90.41, 82.18, and 90.41 nmol/mg in Cantonese sausages with addition of 2.5% FFV, 2.5% DFV and 5.0% DFV, respectively. The 2.5% FFV and 5.0% DFV groups showed similar inhibition effect of sulfhydryl loss. In this study, the sulfhydryl content of the control was much lower than that of FFV and DFV treated groups, suggesting that sulfhydryl loss were retarded by *F. velutipes*. This is likely due to the bioactive compounds of *F. velutipes* that could protect sulfhydryl groups from oxidation by sequestering free radicals (Vaithiyanathan, Naveena, Muthukumar, Girish, & Kondaiah, 2011).

3.5. Effects of *F. velutipes* on color of Cantonese sausages

Color is an important criterion for consumer acceptance of fresh and processed meats (Hamdi et al., 2018). Table 4 shows the L*, a* and b* values of DFV and Cantonese sausages with different levels of FFV and DFV addition. Cantonese sausages incorporated with 2.5% FFV shown the highest L* values ($P < 0.05$) among all groups. The L* value of Cantonese sausages with the addition of 2.5% and 5.0% DFV were significantly decreased compared with control sausages ($P<0.05$), While there was no significant difference between 2.5% and 5.0% DFV groups. Low L* value of sausage with addition of DFV could be explained by the fact that dried *F. velutipes* mushrooms exhibited lower lightness compared to fresh ones because of the nonenzymatic Maillard reaction occurring during the drying process as observed on other mushrooms (Tian et al., 2016). The variation of redness (a*) value recorded for all treatment Cantonese sausages was not significant (P>0.05), due to lower a* value of *F. velutipes* (1.98). This result indicated that addition of FFV and DFV at a certain amount did not affect the a* value of Cantonese sausage, but increasing the added amount of DFV may give a lower a* value, as speculated from the result of addition of 5.0% DFV showing a higher a* value than when 2.5% DFV is added. Sausages added with *F. velutipes* showed significant increased b* values as compared to control samples (P<0.05), partly due to *F. velutipes* being rich in dietary fiber. It was reported that the whiter/yellower fiber added to meat resulted in an increase in b* values (Henning, Tshalibe, & Hoffman, 2016). This phenomenon also is confirmed by the result of dried *F. velutipes* with a higher b* value (21.3). In addition, increasing the amount of DFV slightly increased the b* value of sausages, although the increase was not statistically significant.

3.6. Effects of *F. velutipes* on texture of Cantonese sausages

Texture is not only an indication for the structural integrity of protein matrix but also an important quality parameter for customers. Table 5 shows the textural profile analysis results. Springiness of sausages with the addition of 5.0% DFV were significantly lower than that of control (P<0.05), while there was no significant difference (P>0.05) among 2.5% FFV, 2.5% DFV and control. Cohesiveness of Cantonese sausages with addition of 2.5% DFV and 5.0% FFV were lower than that of control (P>0.05), while there was no significant difference between that of 2.5% FFV addition and control (P>0.05). Cantonese sausages added FFV and DFV at different level had lower hardness and chewiness than control ($p < 0.05$). Higher DFV contents showed the negative effects of decreasing the texture properties. This result may be attributed to the *F. velutipes* being rich in fiber. Previous studies found decreased hardness, gumminess and chewiness in meat products with the addition of dietary fiber (Choe et al., 2018). Han & Bertram (2017) reported that dietary fiber in meat products disrupted the formation of protein-water or protein-protein gel networks and subsequently decreased the gel strength of meat products. In addition, sausages incorporated with 2.5% FFV showed lower texture properties than that with 2.5% DFV. This may be because FFV contained more bioactive enzyme such as proteolytic enzymes which hydrolyzes part of protein into amino acids and/or peptides in the sausages. This result agrees with mushrooms of *Sarcodon aspratus*, *Agaricus bisporus*, and *Lentinula edodes* treated bovine *longissimus dorsi* muscle, suggesting that the proteolytic enzymes in mushroom are able to degrade meat proteins (Lee et al., 2017). Both springiness and cohesiveness of FFV and DFV treatment sausages were slightly lower than the control, but not significantly (P>0.05).

3.7. Sensory evaluation

The sensory panel was convened to assess the effect of the incorporation of FFV and DFV on the color, texture, odor, taste and overall acceptability of the Cantonese sausages (Figure 3). The addition of FFV and DFV gave the lower score on the color and texture properties compared to control. This result was in line with the color and texture observed by instrumental measurements (Table 4 and 5). However, Cantonese sausages treated with 2.5% DFV obtained the highest score of odor and taste, followed by 2.5% FFV and 5.0% DFV, the control gave the lowest score. This may be due to mushrooms enriched in amounts of free amino acids contributing to the taste and flavor properties of sausages (Hospital et al., 2015). As for overall acceptability, the highest score was obtained with the incorporation of 2.5% DFV. This suggests that odor and taste play the significant role in influencing the customer's acceptance.

4. Conclusions

F. velutipes is a commercially important edible mushroom due to its desirable taste and high nutritional components. The present study demonstrates that the addition of *F. velutipes* could be an alternative way to improve the quality of Cantonese sausages by inhibiting lipid and protein oxidation. More important, the addition of *F. velutipes* at appropriate amount could give Cantonese sausages a better overall consumer's acceptance. Although both fresh and dry *F. velutipes* improved the physicochemical and sensory properties of Cantonese sausages, fresh mushroom exhibited better effect when added at the same level. Further studies are needed to adjust the processing conditions in order to control the color and texture of Cantonese sausages.

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References

AOAC. (2005). Official Methods of Analysis of AOAC International. *Association of Official Analysis Chemists International*.

Cai, H., Liu, X., Chen, Z., Liao, S., & Zou, Y. (2013). Isolation, purification and identification of nine chemical compounds from *Flammulina velutipes* fruiting bodies. *Food Chemistry*, 141, 2873–2879.

Carpenter, R.P., D.H. Lyon and T.A. Hasdell, 2000. Guidelines for Sensory Analysis in Food Product Development and Quality Control. (2nd Edn.). Gaithersburg: Aspen Publishers (P. 210).

Chen, Q., Kong, B., Han, Q., Liu, Q., & Xu, L. (2016). The role of bacterial fermentation in the hydrolysis and oxidation of sarcoplasmic and myofibrillar proteins in Harbin dry sausages. *Meat Science*, 121, 196-206.

Cheng, J. R., Liu, X. M., Zhang, Y. S., Zhang, Y. H., Chen, Z. Y., Tang, D. B., & Wang, J. Y. (2017). Protective effects of Momordica grosvenori extract against lipid and protein oxidation-induced damage in dried minced pork slices. *Meat Science,* 133, 26–35.

Cheung, P. C. K. (2010). The nutritional and health benefits of mushrooms. *Nutrition Bulletin,* 35. 292-299.

Choe, J., Lee, J., Jo, K., Jo, C., Song, M., & Jung, S. (2018). Application of winter mushroom powder as an alternative to phosphates in emulsion-type sausages. *Meat Science*, 143, 114-118.

de Torres, C., Díaz-Maroto, M. C., Hermosín-Gutiérrez, I., & Pérez-Coello, M. S. (2010). Effect of freeze-drying and oven-drying on volatiles and phenolics composition of grape skin. *Analytica Chimica Acta*, 660(1-2), 177-182.

Demirkol, M., & Tarakci, Z. (2018). Effect of grape (*Vitis labrusca* L.) pomace dried by different methods on physicochemical, microbiological and bioactive properties of yoghurt. *LWT - Food Science and Technology,* 97, 770-777.

Dong, Y. ru, Cheng, S. jie, Qi, G. hong, Yang, Z. ping, Yin, S. yu, & Chen, G. tang. (2017). Antimicrobial and antioxidant activities of *Flammulina velutipes* polysacchrides and polysacchride-iron(III) complex. *Carbohydrate Polymers,* 161, 26-32.

Gallego, M., Mora, L., Escudero, E., & Toldrá, F. (2018). Bioactive peptides and free amino acids profiles in different types of European dry-fermented sausages. *International Journal of Food Microbiology*, 276(2), 71-78.

Grimrath, A., Berends, P., Rabe, S., Berger, R. G., & Linke, D. (2011). Koji fermentation based on extracellular peptidases of *Flammulina velutipes*. *European Food Research and Technology*, 232(3), 415-424.

Hamdi, M., Nasri, R., Dridi, N., Moussa, H., Ashour, L., & Nasri, M. (2018). Improvement of the quality and the shelf life of reduced-nitrites turkey meat sausages incorporated with carotenoproteins from blue crabs shells. *Food Control*, 91, 148-159.

Han, M., & Bertram, H. C. (2017). Designing healthier comminuted meat products: Effect of dietary fibers on water distribution and texture of a fat-reduced meat model system. *Meat Science*, 133, 159-165.

Henning, S. S. C., Tshalibe, P., & Hoffman, L. C. (2016). Physico-chemical properties of reduced-fat beef species sausage with pork back fat replaced by pineapple dietary fibres and water. *LWT - Food Science and Technology*, 74, 92-98.

Hospital, X. F., Carballo, J., Fernandez, M., Arnau, J., Gratacos, M., & Hierro, E. (2015). Technological implications of reducing nitrate and nitrite levels in dry-fermented sausages:

Typical microbiota, residual nitrate and nitrite and volatile profile. *Food Control*, 57, 275-281.

Ioannis G., (2014). Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals. *Current Opinion in Biotechnology*, 26, 162–173.

Jiang, J., & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, 120, 107-117.

Kaewprachu, P., Osako, K., Benjakul, S., Suthiluk, P., & Rawdkuen, S. (2017). Shelf life extension for Bluefin tuna slices (*Thunnus thynnus*) wrapped with myofibrillar protein film incorporated with catechin-Kradon extract. *Food Control*, 79, 333-343.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, 113(1), 9-16.

Kang, G. H., Kim, S. H., Kim, J. H., Kang, H. K., Kim, D. W., Seong, P. N., & Kim, D. H. (2012). Effect of *Flammulina velutipes* on spent-hen breast meat tenderization. *Poultry Science*, 91, 232–236.

Kang, L. Z., Zeng, X. L., Ye, Z. W., Lin, J. F., & Guo, L. Q. (2014). Compositional analysis of the fruiting body of transgenic *Flammulina velutipes* producing resveratrol. *Food Chemistry*, 164, 211-218.

Lawless, H. T., & Heymann, H. (2010). Sensory Evaluation of Food and Practices. In *Sensory evaluation of food - principles and practices*. (2nd ed.). New York: Springer (Chapter 10).

Lee, K. H., Kim, H. K., Kim, S. H., Kim, K. H., Choi, Y. M., Jin, H. H., Ryu, Y. C. (2017). Effects of mushroom extract on textural properties and muscle protein degradation of bovine *longissimus dorsi* muscle. *Bioscience, Biotechnology and Biochemistry*, 81(3), 558-564.

Leung, M. Y. K., Fung, K. P., & Choy, Y. M. (1997). The isolation and characterization of an immunomodulatory and anti-tumor polysaccharide preparation from *Flammulina velutipes*. *Immunopharmacology*, 35(3), 255-263.

López-López, I., Cofrades, S., Ruiz-Capillas, C., & Jiménez-Colmenero, F. (2009). Design and nutritional properties of potential functional frankfurters based on lipid formulation, added seaweed and low salt content. *Meat Science*, 83(2), 255-262.

Mendoza, E., García, M. L., Casas, C., & Selgas, M. D. (2001). Inulin as fat substitute in low fat, dry fermented sausages. *Meat Science*, 57(4), 387-393.

Olmedilla-Alonso, B., Jiménez-Colmenero, F., & Sánchez-Muniz, F. J. (2013). Development and assessment of healthy properties of meat and meat products designed as functional foods. *Meat Science*, 95(4), 919-930.

Pahila, J., Kaneda, H., Nagasaka, R., Koyama, T., & Ohshima, T. (2017). Effects of ergothioneine-rich mushroom extracts on lipid oxidation and discoloration in salmon muscle stored at low temperatures. *Food Chemistry*, 233, 273-281.

Rosli, W., & Maihiza, N. (2015). The ability of oyster mushroom in improving nutritional composition, β-glucan and textural properties of chicken frankfurters. *International Food Research Journal*, *22*(1), 311–317.

Sánchez-Macías, D., Barba-Maggi, L., Morales-delaNuez, A., & Palmay-Paredes, J. (2018). Guinea pig for meat production: A systematic review of factors affecting the production, carcass and meat quality. *Meat Science*, 143, 165-176.

Seiquer, I., Palma-Granados, P., Haro, A., Lara, L., Lachica, M., Fernández-Fígares, I., & Nieto, R. (2019). Meat quality traits in longissimus lumborum and gluteus medius muscles from immunocastrated and surgically castrated Iberian pigs. *Meat Science*, *150*, 77–84.

Shin, H. G., Choi, Y. M., Kim, H. K., Ryu, Y. C., Lee, S. H., & Kim, B. C. (2008). Tenderization and fragmentation of myofibrillar proteins in bovine *longissimus dorsi* muscle using proteolytic extract from Sarcodon aspratus. *LWT - Food Science and Technology*, 41, 1389–1395.

Tian, Y., Zhao, Y., Huang, J., Zeng, H., & Zheng, B. (2016). Effects of different drying methods on the product quality and volatile compounds of whole shiitake mushrooms. *Food Chemistry*, 197(Part A), 714-722.

Turgut, S. S., Soyer, A., & Işıkçı, F. (2016). Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage. *Meat Science*, 116, 126-132.

Vaithiyanathan, S., Naveena, B. M., Muthukumar, M., Girish, P. S., & Kondaiah, N. (2011). Effect of dipping in pomegranate (*Punica granatum*) fruit juice phenolic solution on the shelf life of chicken meat under refrigerated storage (4°C). *Meat Science*, 88(3), 409-414.

Van Hecke, T., Ho, P. L., Goethals, S., & De Smet, S. (2017). The potential of herbs and spices to reduce lipid oxidation during heating and gastrointestinal digestion of a beef product. *Food Research International*, 102, 785-792.

Wang, X., Zhou, P., Cheng, J., Chen, Z., & Liu, X. (2018). Use of straw mushrooms (*Volvariella volvacea*) for the enhancement of physicochemical, nutritional and sensory profiles of Cantonese sausages. *Meat Science*, *146*(April), 18–25.

WCRF/AICR. (2007). Food, nutrition, physical activity, and the prevention of cancer: a global perspective.

WHO. (2003). Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. WHO Technical Report Series (P.916).

Wu, M., Luo, X., Xu, X., Wei, W., Yu, M., Jiang, N., & Fei, X. (2014). Antioxidant and immunomodulatory activities of a polysaccharide from *Flammulina velutipes*. *Journal of Traditional Chinese Medicine*, 34(6), 733-740.

Yi, C., Sun, C., Tong, S., Cao, X., Feng, Y., Firempong, C. K., Yu, J. (2013). Cytotoxic effect of novel *Flammulina velutipes* sterols and its oral bioavailability via mixed micellar nanoformulation. *International Journal of Pharmaceutics*, 448(1), 44-50.

Table 1 Formulation for manufacturing Cantonese sausages with different levels of FFV and DFV.

	Control	Different treatments of *F. velutipes* (%, w/w meat)		
		2.5% FFV	2.5% DFV	5.0% DFV
Lean pork (g)	800	800	800	800
Back fat (g)	200	200	200	200
Salt (g)	23	23	23	23
Sugar (g)	120	120	120	120
Chinese liquor (g)	20	20	20	20
MSG (g)	2	2	2	2
sodium nitrite (g)	0.2	0.2	0.2	0.2
FFV[a] (g)	-	25	-	-
DFV[a] (g)	-	-	25	50

[a] The added amount was calculated according to dry mushroom weight.

Table 2 Nutritional compositions of control, 2.5% FFV, 2.5% DFV and 5.0% DFV Cantonese sausages

	Control	2.5% FFV	2.5% DFV	5.0% DFV	DFV
Moisture (%)	18.48±0.20[a]	18.57±0.11[a]	19.11±0.32[ab]	19.87±0.64[b]	5.17±0.08
Protein (%)	27.27±0.49[ab]	28.28±0.08[b]	26.43±0.57[a]	26.73±0.19[ab]	22.16±0.21
Fat (%)	28.87±0.78[c]	26.18±0.50[b]	24.71±0.25[b]	22.08±0.21[a]	0.98±0.05
Ash (%)	6.30±0.10[a]	6.32±0.04[a]	6.12±0.02[a]	6.21±0.01[a]	6.05±0.17

Data expressed as mean ± standard errors;

Different letters in the same row indicate significant differences (P<0.05).

Table 3 Effects of FFV and DFV on free amino acids of Cantonese sausages

Free amino acid (mg/100g)	Control	2.5% FFV	2.5% DFV	5.0% DFV	DFV	FFV
Taste FAA						
Asp	0.39±0.05^{a}	n.d.	0.64±0.02^{b}	0.96±0.01^{c}	n.d.	n.d.
Ser	15.85±0.87^{a}	102.34±14.11^{bc}	90.24±1.54^{b}	119.25±1.79^{c}	136.34±3.07	127.53±3.88
Glu	206.79±1.71^{a}	308.33±42.19^{b}	252.87±4.25^{ab}	320.57±2.00^{b}	593.20±10.21	1286.98±57.33
Gly	26.55±0.79^{a}	74.33±9.72^{bc}	61.06±1.23^{b}	87.79±2.84^{c}	131.79±2.41	121.35±1.22
Ala	77.63±3.65^{a}	191.78±25.57^{b}	170.58±4.27^{b}	242.99±13.58^{c}	279.20±8.32	322.27±1.73
Pro	14.70±0.59^{a}	56.72±8.16^{c}	37.23±1.08^{b}	60.41±6.20^{c}	93.24±1.74	53.11±1.36
∑Taste FAA	341.91±5.33^{a}	612.62±91.59^{bc}	831.97±11.3^{b}	733.50±20.22^{c}	1233.77±24.02	1911.24±61.72
Essential FAA						
Val $^{\#}$	24.69±1.12^{a}	99.96±13.98^{bc}	80.84±2.58^{b}	124.63±5.84^{c}	138.35±2.58	105.37±2.24
Met $^{\#}$	12.34±0.18^{a}	52.07±7.40^{bc}	42.39±1.37^{b}	60.89±3.26^{c}	8.97±0.28	7.69±0.22
Ile $^{\#}$	20.29±0.63^{a}	73.03±10.46^{bc}	60.33±1.98^{b}	87.54±2.48^{c}	105.56±1.61	61.44±3.00
Leu	32.92±1.52^{a}	151.27±21.17^{bc}	120.43±3.80^{b}	183.33±8.62^{c}	167.80±3.23	99.79±3.59
Phe $^{\#}$	20.43±1.36^{a}	92.72±13.83^{b}	81.79±3.06^{b}	124.50±5.23^{c}	351.33±5.71	339.91±10.86
Lys	42.26±2.98^{a}	193.04±31.75^{bc}	150.54±6.10^{b}	219.89±13.70^{c}	214.74±5.74	350.89±14.15
∑Essential FAA	152.93±7.79^{a}	662.09±98.59^{bc}	536.32±18.89^{b}	800.78±39.12^{c}	986.74±18.58	965.09±34.05
Other FAA						
α-AAA	4.16±0.18^{a}	15.03±2.19^{bc}	12.41±0.53^{b}	18.54±0.55^{c}	63.49±2.13	n.d.
Tau	78.21±2.45^{a}	69.90±6.06^{a}	75.46±1.31^{a}	74.61±1.71^{a}	n.d.	n.d.
α-ABA	2.32±0.03^{a}	6.39±1.21a^{b}	6.99±0.15^{b}	10.83±0.30^{c}	17.21±0.54	24.98±4.04
Cys	1.82±0.19^{a}	3.92±0.70^{b}	4.24±0.17^{b}	5.18±0.26^{b}	291.07±8.74	222.45±11.08
Cysta	1.45±0.06^{a}	4.94±0.98^{b}	7.37±0.36^{c}	11.62±0.04^{d}	112.73±1.68	52.41±2.37
Tyr	17.52±0.85^{a}	69.24±10.3^{bc}	56.22±2.08^{b}	88.10±7.96^{c}	289.88±5.55	271.06±9.54
β-Ala	6.33±0.54^{a}	23.11±3.71^{c}	17.11±0.52^{ab}	31.16±6.65^{c}	20.83±1.17	15.14±1.07
β-AIBA	0.86±0.12^{a}	9.58±1.56^{b}	9.86±0.31^{b}	16.87±2.09^{c}	21.47±2.10	5.12±0.42
γ-ABA	1.53±0.06^{a}	52.35±6.76^{b}	65.61±1.50^{b}	108.02±8.70^{c}	396.96±4.28	48.92±1.82
Hylys	0.85±0.03^{a}	4.07±0.53^{bc}	2.73±0.9^{b}	4.94±0.11^{c}	3.78±0.05	n.d.
Orn	1.57±0.12^{a}	25.75±4.14^{b}	27.54±0.11^{b}	50.81±3.13^{c}	542.21±13.52	898.92±35.18
His	12.69±0.86^{a}	54.58±9.51^{bc}	43.81±2.20^{b}	65.17±4.27^{c}	166.92±3.77	226.32±8.99
Arg	27.55±2.14^{a}	129.66±22.12^{bc}	96.88±4.85^{b}	143.14±8.27^{c}	110.83±3.12	130.85±5.78
Total FAA	651.70±20.84^{a}	1864.63±268.19^{bc}	1575.18±44.24^{b}	2262.29±104.77^{c}	4257.88±83.35	4772.50±169.35

Data expressed as mean ± standard errors; Different letters in the same row indicate significant differences ($P<0.05$);

n.d. : Not detected.

Abbreviation: α-AAA (α-aminoacetic acid), α-ABA (α-aminobutyric acid), Tau (taurine), Cysta (cystathionine), β-AIBA (β-aminoisobutyric acid), Hylys (hydroxylysine), Orn (ornithine).

Table 4 Color comparison of Cantonese sausage prepared with various levels of FFV and DFV.

	L*	a*	b*
Control	46.62±0.30[b]	11.10±0.18[a]	15.21±0.14[c]
2.5% FFV	49.21±0.92[a]	11.42±0.33[a]	16.22±0.50[ab]
2.5% DFV	44.37±0.24[c]	11.09±0.65[a]	16.82±0.26[ab]
5.0% DFV	43.66±0.74[c]	10.40±0.60[a]	17.93±0.99[a]
DFV	39.31±0.56	1.98±0.14	21.28±0.27

Data expressed as mean ± standard errors.

Different letters in the same row indicate significant differences (P<0.05).

Table 5 Textural profile of Cantonese sausage prepared with various levels of FFV and DFV

	Hardness (N)	Springiness (cm)	Cohesiveness (N*S)	Chewiness (N*cm)
Control	8813±109[a]	0.934±0.017[a]	0.710±0.023[a]	6080±99[a]
2.5% FFV	4768±96[c]	0.889±0.019[ab]	0.574±0.019[b]	3057±147[c]
2.5% DFV	7569±161[b]	0.875±0.009[ab]	0.614±0.052[a]	4774±88[b]
5.0% DFV	5945±72[c]	0.826±0.021[b]	0.520±0.012[b]	3361±64[c]

Data expressed as mean ± standard errors;

Different letters in the same row indicate significant differences (P<0.05).

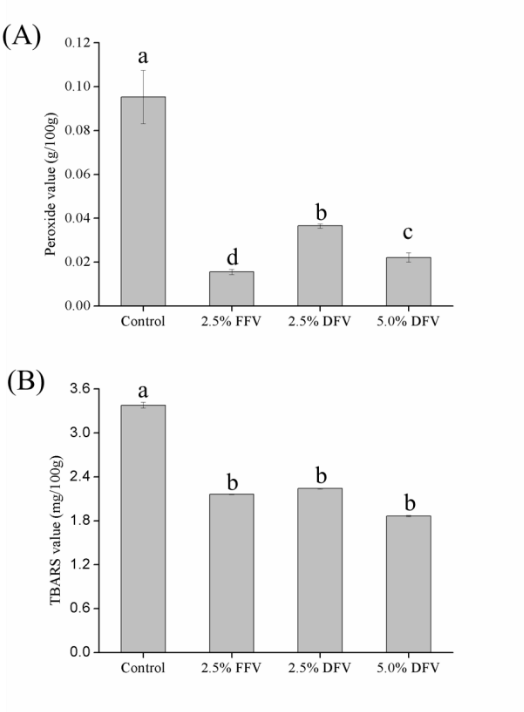

Figure 1 Effects of FFV and DFV on peroxide value (A) and TBARS value (B) of Cantonese sausages. Error bars refer to the standard errors obtained from triplicate measurements. Means with different letters indicate significant differences (P < 0.05).

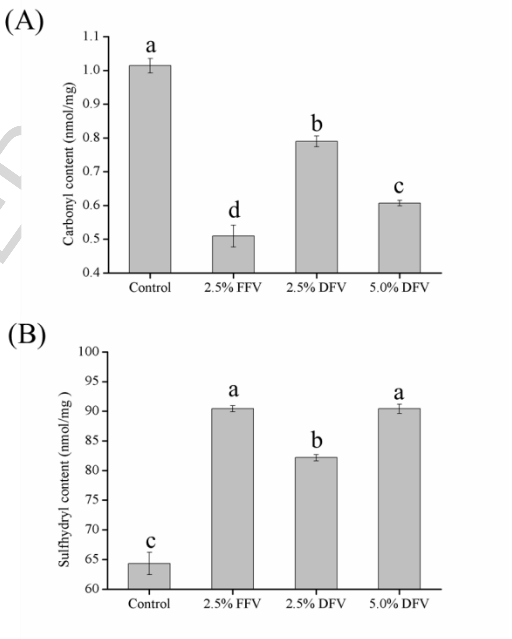

Figure 2 Effects of FFV and DFV on carbonyl (A) and sulfhydryl contents (B) of Cantonese sausages. Error bars refer to the standard errors obtained from triplicate measurements. Means with different letters indicate significant differences (P < 0.05).

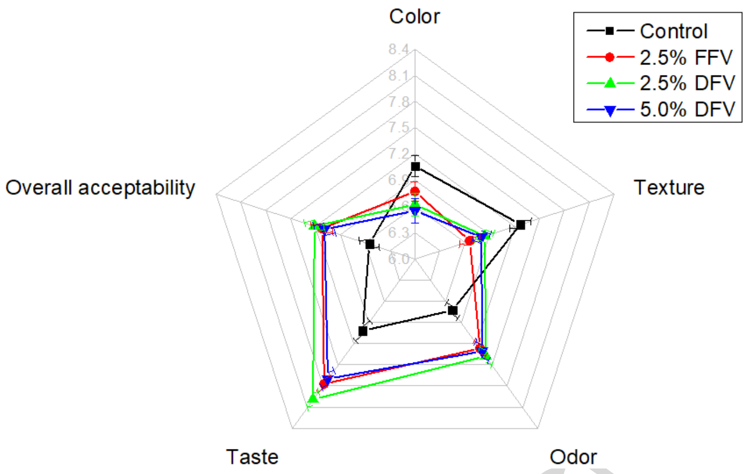

Figure 3 Sensory properties of Cantonese sausage prepared with various levels of FFV and DFV

Highlights

- The quality and sensory characteristics of sausages can be improved by addition of *F. velutipes*.
- Addition of *F. velutipes* significantly decreased fat content of Cantonese sausages.
- Lipid and protein oxidation can be effectively inhibited by the addition of *F. velutipes*.
- Sausages incorporated with 2.5% Dried *F. velutipes* showed the best overall sensory acceptance.