Application of *Auricularia cornea* as a Pork Fat Replacement in Cooked Sausage

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Abstract: The effect of *Auricularia cornea* (AC) as an alternative for pork fat on the physico-chemical properties and sensory characteristics of cooked sausage were evaluated. The results indicated that replacement of pork fat with AC led to a significant increase in the protein, ash, moisture, cooking loss, water holding capacity, springiness, and chewiness, especially isoleucine, leucine, proline, palmitic, palmitoleic, oleic, and arachidonic acids of the sausages. In contrast, AC reduced the level of fat (12.61%–87.56%) and energy (5.76%–56.40%) of the sausages. In addition, AC led to the mild lightness, yellowness, whiteness, and soft texture, while it did not affect the water activity of the sausages. From the sensory point of view, all sausages were judged acceptable, and the substitution of 75% of pork fat by AC exhibited best sensory characteristics. In a word, AC is a promising food to partially replace the pork fat in sausages.

Keywords: mushroom; fat replacement; meat product

1. Introduction

The World Health Organization (WHO) and Drug Administration (FDA) recommended a reduction in ingestion of total fat and saturated fatty acids (SFAs) [1], since recent reports have shown that high intake of SFAs will increase the risk of several chronic diseases, such as obesity, hypertension, colon cancer, cardiovascular diseases, and coronary heart diseases [2]. However, the popular sausage products have a high-fat content (20%–30%) and characterized by a high proportion of SFAs [3]. Thus, it is necessary to develop healthier nutritional sausages with lower animal fat.

Some healthy ingredients have been used to replace animal fat in meat products, resulting in better nutritional properties [3]. *Camellia* oil gel [4], interesterified palm kernel oil [5], hydroxypropyl methylcellulose oleogel [6], hazelnut [7], pre-emulsified perillacanola oil [8], and different vegetable oils have been used to partially replace animal fat in meat products [9]. Certainly, two materials also can be used to replace the fat, such as whey proteins and sodium dodecylsulfate [2], konjac gel with vegetable powders [10], double emulsions with olive leaves extract [1], cellulose nanofibers and its palm oil pickering emulsion [11], canola oil hydrogels and organogels [12], inulin-based emulsion-filled gel [13], and carboxymethyl cellulose and inulin [14]. Quite a few studies had successfully achieved healthier products, while some fat replacer not only impaired the texture, but also accelerated lipid oxidation reactions with reduction of shelf life and loss of sensorial and nutritional values [6]. New fat substitutes should be developed.

*Auricularia cornea* (AC), also called “Yu Mu Er” in Chinese, is a pure white strain of the variant of *Auricularia cornea* species, which has been artificially cultivated by the team...
of Yu Li in Jilin Agricultural University and commercialized in China [15]. It is crystal clear appearance, tender, delicious taste, and rich in nutrients, including polysaccharides, dietary fiber, amino acids, and various trace elements [16]. The polysaccharides extracted from AC have antioxidant, hepatoprotective, and anti-tumor activities [17]. Thus, it exhibits potential to be a fat replacer.

The objective of the present study was to evaluate the effect of AC as an alternative for pork fat on the proximate composition, water activity, pH, color, cooking loss, water holding capacity, textural profile, free amino acids, free fatty acids, and sensory characteristics of cooked sausage.

2. Materials and Methods

2.1. Materials

The pork lean meat (protein: 20.16%; fat: 2.57%; moisture: 71.11%; ash: 1.18%) and back fat (protein: 0.54%; fat: 91.01%; moisture: 7.26%; ash: 0.17%) were obtained from Jilin Huazheng Agriculture and Animal Husbandry Development Co. Ltd. (Changchun, China) and stored at $-20^\circ$C until use. Dry Auricularia cornea was supplied by Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi. All spices (salt; sugar; wheat; carrageen; isolated soy protein; dry starch; ice) were obtained from the local market. All the additives and chemical reagents were purchased from Sichuan Jinshan Pharmaceutical Co. Ltd. (Guangyuan, China) and Beijing Beihua Co. Ltd. (Beijing, China), respectively.

2.2. Sausages Formulation and Processing

The lean pork meat was minced and the back fat was cut into approximately 1.5 cm cubes. Dry Auricularia cornea was immersed in water at 40 $^\circ$C for 30 min, then cut into 0.5 cm slices. The sausages were prepared using the formulations that are shown in Table 1. Differently, the control sausage formulation was made with pork fat, and the other four formulations (AC25, AC50, AC75, and AC100) were prepared with substitution of 25%, 50%, 75%, and 100% of pork fat by AC, respectively. Each sausage (Control, AC25, AC50, AC75, and AC100) was added according to the formula and the ingredients were homogenized by the blender (German K + G wetter touch screen) for 140 s. The sausage was roasted at 68 $^\circ$C for 30 min, steamed at 80 $^\circ$C for 50 min, and smoked at 50 $^\circ$C for 150 min. The three processes were completed by an electric heating flue gas furnace (Zhucheng Yizhong Machinery Co., LTD., Weifang, China). After cooling, the samples were individually packaged in polyethylene bags using a vacuum packaging machine (Zhucheng Yizhong Machinery Co., LTD., Weifang, China) and stored at 4 ± 1 $^\circ$C for subsequent analysis.

Table 1. Formulations of sausages with alternative of pork fat at Auricularia cornea (AC).

| Ingredients (%) | Control | AC25 | AC50 | AC75 | AC100 |
|-----------------|---------|------|------|------|-------|
| Pork lean meat  | 49      | 49   | 49   | 49   | 49    |
| Pork back fat   | 21      | 15.75| 10.5 | 5.25 | 0     |
| AC              | 0       | 5.25 | 10.5 | 15.75| 21    |
| Salt            | 1.5     | 1.5  | 1.5  | 1.5  | 1.5   |
| Sugar           | 1       | 1    | 1    | 1    | 1     |
| White pepper    | 0.2     | 0.2  | 0.2  | 0.2  | 0.2   |
| Carrageenan     | 0.3     | 0.3  | 0.3  | 0.3  | 0.3   |
| Isolated soy protein | 2.8 | 2.8  | 2.8  | 2.8  | 2.8   |
| Dry starch      | 4.2     | 4.2  | 4.2  | 4.2  | 4.2   |
| Ice             | 20      | 20   | 20   | 20   | 20    |
| Total           | 100     | 100  | 100  | 100  | 100   |

Notes: Control (0%), AC25 (25%), AC50 (50%), AC75 (75%), AC100 (100%) were substitution of pork fat by AC, respectively.
2.3. Proximate Composition and Energy Value

The proximate composition of cooked sausages was detected using the Association of Official Analytical Chemists (AOAC, 2005). Energy value was calculated based on 9 kcal/g for fat, 4 kcal/g for protein and carbohydrate [18].

2.4. Water Activity and pH

The water activity of sausages was measured by a water activity meter (Rotronic, Bassersdorf, Switzerland). The pH of sausage was determined on pH meter (Mettler Toledo, Columbus, OH, USA).

2.5. Color

The color parameters of different sausages were determined by a previously described method [19].

2.6. Cooking Loss and Water Holding Capacity

Cooking loss of sausages was conducted according to [11]. Raw sausage samples (50 g) were cooked at 80 °C for 50 min, then calculated as follows:

\[
\text{Cooking loss } (\%) = \left( \frac{m_1 - m_2}{m_1} \right) \times 100\% \tag{1}
\]

where m1 is the weight of raw sausages and m2 is the weight of cooked sausages.

The water holding capacity (WHC) was detected according to [20]. Sausage samples were respectively centrifuged for 30 min at 12,000 × g centrifugal force, then calculated as follows:

\[
\text{WHC} = \left( \frac{W_2}{W_1} \right) \times 100\% \tag{2}
\]

where W1 is the weight of the sample before centrifugation and W2 is the weight of the sample after centrifugation.

2.7. Textural Profile Analysis

TPA was performed by using a texture analyzer (TMA-Pro, FTC, Washington, DC, USA) according to [11], with some slight modifications. Sausages were cut into 1.0 cm height and 1.5 cm diameter. The detection speed was 60 mm/min and minimum force 0.8 N. The characteristics of sausage were hardness, cohesiveness, springiness, gumminess, and chewiness. All samples were performed in triplicate at room temperature, and the average value was taken.

2.8. Free Amino Acids

The amino acids content was detected by the method according to [21], with slight modifications. The sample (0.2 g) was hydrolyzed by 6 mol/L HCl (10 mL) for 24 h at 110 °C. After cooling at room temperature, a hydrolyzed sample was volumed to 50 mL. 10 mL from the 50 mL hydrolysate was taken and dried, the dried sample adding 0.1 mol/L HCl solution to 10 mL. The solution was filtered through a 0.22 µm water membrane. After filtration, the filtrate (500 µL) and internal standard solution (50 µL) were mixed and derived. The derivative solution (2 µL) was injected into Liquid Chromatography (20AT-PDA (Diode Array Detector) Detector, Shimazu, Kyoto, Japan). The measurement conditions were as follows: chromatographic column (C18: Ajs-01 amino acid special analytical column, 3 µm, 4.6 mm × 150 mm), detection wavelength, 338 nm; column temperature, 50 °C. Elution gradient and flow rate are as follows: time: 0 s, 6 s, 8 s, 10 s, 23 s, 30 s, 31 s, 34 s, 35 s, 38 s; mobile phase B%: 5, 10, 16, 40, 50, 100, 100, 55; flow rate (mL/min): 1.6, 1.6, 1.6, 1.3, 1.0, 1.6, 1.6, 1.6, 1.6.
2.9. Free Fatty Acids

Free fatty acid was detected by a method previously published according to [22], with slight modifications. Determination of fatty acid methyl esters (FAME) was performed using a GC/MS system equipped with a GC-7 Agilent HP-88 capillary column (60 m × 0.25 mm × 0.2 µm). The temperature profile of the oven was 100 °C for 13 min followed by increasing at 10 °C/min to 180 °C for 6 min, then increasing at 1 °C/min to 200 °C for 20 min, and then increasing at 4 °C/min to 230 °C for 10.5 min. Injector and detector temperatures were set to 270 and 280 °C, respectively. The conditions applied for gas chromatography were nitrogen as the carrier gas at a flow of 1.0 µL/min. Fatty acids were identified and quantified based on chromatographic retention times using reference standard Supelco 37 component FAME mix (Sigma Aldrich Chemical Co., St. Louis, MO, USA). Results obtained were presented as percentage of total fatty acids.

2.10. Sensory Evaluation

A thirty-member panel from the Departments of College of Food Science and Engineering at Jilin Agricultural University have evaluated the sensory attributes of samples. Sensorial analysis has used the method described by [23], with some modifications. Unsalted crackers and water were offered to clean the palates and remove residual flavors between samples. The samples were reheated using a microwave oven, sliced to 2 cm thick, placed on plates and provided to panelists. The appearance, odor, taste, texture, and overall acceptability of the sausage samples were evaluated with a 10-hedonic scale from 1 (dislike extremely) to 10 (like extremely).

2.11. Statistical Analysis

One-way ANOVA was used to determine statistical significance in the data compared the sausages on the proximate composition, water activity, pH, color, cooking loss, water holding capacity, textural profile, free amino acids, free fatty acids, and sensory characteristics of cooked sausage. Duncan multiple range test was carried out to compare the mean between the two groups to determine which groups had significant differences compared with other groups $(p < 0.05)$. All data were expressed as mean ± standard error.

3. Results

3.1. Proximate Composition and Energy Value

The results of proximate composition and energy value analysis of sausages are listed in Table 2. Significant differences $(p < 0.05)$ were observed among the protein, fat, moisture, ash contents, carbohydrate, and energy value between control and fat replacement group (AC25, AC50, AC75, and AC100). The control shown the lowest protein content, close to the 12.73% protein content in model sausages as reported by [24], but lower than the protein content (13.77%) in frankfurters as described by [25]. As the proportion of AC increased, the protein content in the sausages increased by 1.42%–14.31%. These changes were attributed to the protein in AC.

The fat levels in the replacement sausages significantly $(p < 0.05)$ decreased by 12.61%–87.56%, compared with those of the control. The fat content of AC100 was similar to those of the sausage with fried Pleurotus eryngii as fat replacements [11], but lower than those of frankfurters with porcine plasma protein hydrolysates and oxidized tannic acid to partially replace pork fat in [25]. It was because the replacement AC is extremely low in fat. The moisture levels of the sausages were significantly $(p < 0.05)$ affected by the increase of AC concentration. Compared to the control, the moisture contents of AC25, AC50, AC75, and AC100 increased by 1.62%, 16.74%, 26.08%, and 31.64%, respectively. The reason was that the moisture content of the AC was higher after water treatment.

In terms of ash content, the replacement group was notably $(p < 0.05)$ higher than the control. The ash content of sausages was from 3.12% to 3.57%. The consequences were approximate to the value in frankfurters with phenolic compounds in emulsion gel-based delivery systems as animal fat replacers, which was reported by [18], but higher than those


in Bologna sausage and Toscana sausage with partial substitution of pork fat with canola oil, as determined by [5] and [26], respectively.

Table 2. Proximate composition (%), energy value (kcal/100 g of the product), water activity, pH, cooking loss and water holding capacity of sausages with replacement of pork fat by Auricularia cornea (AC).

| Parameters               | Control | AC25 | AC50 | AC75 | AC100 |
|--------------------------|---------|------|------|------|-------|
| Protein                  | 12.65 ± 0.14 a | 12.83 ± 0.07 a | 13.16 ± 0.21 b | 13.47 ± 0.02 b | 14.46 ± 0.02 c |
| Fat                      | 17.77 ± 0.65 c | 15.53 ± 0.05 d | 8.34 ± 0.05 c | 5.47 ± 0.04 b | 2.21 ± 0.27 a |
| Moisturer                | 53.69 ± 0.09 a | 54.56 ± 0.06 b | 62.68 ± 0.08 c | 67.69 ± 0.04 d | 70.68 ± 0.08 e |
| Ash                      | 3.12 ± 0.01 a | 3.22 ± 0.01 b | 3.53 ± 0.03 c | 3.55 ± 0.05 c | 3.57 ± 0.06 c |
| Carbohydrate             | 12.77 ± 0.09 b | 13.86 ± 0.05 a | 12.29 ± 0.01 c | 9.82 ± 0.01 d | 9.08 ± 0.02 e |
| Energy value             | 261.61 | 246.53 | 176.86 | 142.39 | 114.05 |
| Water activity           | 0.99 ± 0.01 a | 0.98 ± 0.00 a | 0.98 ± 0.00 a | 0.98 ± 0.00 a | 0.98 ± 0.00 a |
| pH                       | 6.33 ± 0.03 b | 6.30 ± 0.01 b | 6.23 ± 0.03 a | 6.21 ± 0.02 a | 6.20 ± 0.01 a |
| Cooking loss             | 5.13 ± 0.12 a | 11.73 ± 0.07 b | 12.79 ± 0.08 c | 13.69 ± 0.08 d | 14.68 ± 0.09 e |
| Water holding capacity   | 82.08 ± 0.1 a | 83.27 ± 0.01 b | 86.45 ± 0.24 c | 88.49 ± 0.36 d | 91.16 ± 0.51 e |

Notes: Values are given as mean ± standard error. Different letters in the same row indicate significant differences (p < 0.05). Control (0%), AC25 (25%), AC50 (50%), AC75 (75%), AC100 (100%) substitution of pork fat by AC, respectively.

Energy values of the control (261.61 kcal/100 g) was significantly (p < 0.05) higher than that of replacement groups, decreasing by 5.76%–56.40%. The energy value of the AC50 were similar with those obtained by [11]—that is 171.8 kcal/100 g of the pork sausages using deep-fried Pleurotus eryngii as replacements for pork fat. The energy level in AC75 and AC100 sausages were lower than those found by [18]—that is 174–196 kcal/100 g of frankfurters with phenolic compounds in emulsion gel-based delivery systems as animal fat replacers, and [27]—that is 146.24–198.47 kcal/100 g of low-fat burger with high beta-glucans content and oat-hull-based ingredient as a fat replacer.

3.2 Water Activity and pH

The water activity and pH of cooked sausage samples are shown in Table 2. There were no significant differences (p > 0.05) in water activity levels among all the samples, suggesting that AC did not affect the bound water content in the sausages. The consequences were similar to the values in hot-dog style sausages with pork skin-based emulsion gels as animal fat replacers. The authors of [28] also reported that hydrolyzed collagen or pork skin/green banana flour gel used as a fat replacer in frankfurter-type sausages or Bologna sausages did not influence the water activity.

As the proportion of AC increased, the pH of sausages decreased by 0.47%–2.05%. These changes were put down to the lower pH of AC. The pH of the control was approximate to that obtained by [29], and no significant differences (p > 0.05) were found among the AC50, AC75, and AC100. This decline trend was approximate to the trend observed by [24] in the sausage with Lentinula edodes as the pork lean meat replacer, [11] in pork back sausages with fat replaced by fried Pleurotus eryngii, and [29] in frankfurter-type sausages with hydrolyzed collagen as replacements for fat.

3.3 Color

The influence of AC on the color of sausages are shown in Table 3. L*, a*, b*, ∆E*, and whiteness value of the sausages notably (p < 0.05) improved with an increase in AC. These changes exhibited that color of the sausages became slight lightness, yellowness, and whiteness, which was put down to the larger amounts of AC and smaller amount of pork fat in sausages. As the proportion of AC increased, a* value decreased significantly (p < 0.05), suggesting that the redness of the replacement group was less than control due to the AC with a white crystal-clear appearance. Similar observations in L* and b* were found by [11], who used boiled Pleurotus eryngii to substitute the pork back fat in sausages.
Table 3. Color and textural profile of sausages with substitution of pork fat by Auricularia cornea (AC).

| Parameters       | Control  | AC25     | AC50     | AC75     | AC100    |
|------------------|----------|----------|----------|----------|----------|
| L*               | 54.37 ± 1.80 a | 55.43 ± 1.13 a,b | 56.44 ± 0.30 a,b | 57.43 ± 0.67 b | 59.68 ± 0.84 c |
| A*               | 16.06 ± 1.74 b | 15.25 ± 0.37 b | 14.28 ± 1.21 a,b | 14.31 ± 0.40 a,b | 13.23 ± 0.45 a |
| B*               | 15.08 ± 0.36 a | 16.91 ± 0.62 b,a | 17.33 ± 0.84 b,c | 17.03 ± 0.26 b,c | 17.98 ± 0.42 c |
| ΔE*              | 58.66 ± 2.24 a | 59.92 ± 1.42 a,b | 60.74 ± 0.68 a,b | 61.58 ± 0.28 b,c | 63.71 ± 0.64 c |
| Whiteness        | 49.93 ± 5.25 a | 49.95 ± 1.47 a,b | 50.99 ± 0.70 a,b | 51.97 ± 0.31 b,c | 53.91 ± 0.52 c |
| Hardness (N)     | 155.63 ± 11.14 b | 141.17 ± 5.97 b,a | 107.23 ± 8.15 a | 102.70 ± 6.48 a | 102.86 ± 4.36 a |
| Springiness (mm) | 3.50 ± 0.22 a | 3.58 ± 0.36 a,b | 3.63 ± 0.59 a,b | 3.88 ± 0.22 a,b | 4.23 ± 0.42 b |
| Gumminess (N)    | 85.23 ± 13.31 c | 54.33 ± 7.26 b | 28.50 ± 8.74 a | 25.67 ± 4.48 a | 23.43 ± 5.87 a |
| Chewiness (N)    | 248.49 ± 8.60 a | 253.13 ± 7.85 a | 255.63 ± 7.09 a | 262.12 ± 6.92 b | 268.59 ± 8.94 b |
| Cohesiveness     | 0.55 ± 0.03 c | 0.39 ± 0.07 b | 0.27 ± 0.03 a | 0.26 ± 0.01 a | 0.23 ± 0.06 a |

Notes: Values are given as mean ± standard error. Different letters in the same row indicate significant differences (p < 0.05). Control (0%), AC25 (25%), AC50 (50%), AC75 (75%), AC100 (100%) substitution of pork fat by AC, respectively.

3.4. Cooking Loss and Water Holding Capacity

Cooking loss is a crucial factor for juiciness and is associated with water or fat binding capacities during the heating process [25]. The cooking loss of sausages is presented in Table 2. The substitution of pork fat by AC exerted a significant effect (p < 0.05) on cooking loss (Figure 1). The sausages with low-fat replacer contents showed higher cooking loss. Cooking loss in of replacement group was 2.29–2.86 folds reference to that in the control. Because the AC found much more water than in pork fat. Another reason may be that the capacity of AC to retain water during cooking was decreased. AC100 showed the highest cooking loss, which was significantly higher than that of the control (p < 0.05). Similar observations were found by [11] who used boiled and deep-fried Pleurotus eryngii instead of fat in pork sausage, and [24] who used Lentinula edodes as a pork lean meat replacer in sausage.

The water holding capacity of the sausages is also shown in Table 4. The WHC increased as the percentage of AC increased. The WHC of the control was significant (p < 0.05) lower than that of all replacement groups. AC25, AC50, AC75, and AC100 were increased by 1.45%, 5.32%, 7.81%, and 11.06%, compared to the control, respectively. This
phenomenon might be that the AC could help hold not only water but also fat to prevent their loss during cooking.

Table 4. Free amino acids (expressed as g/100 g of sausage) profile of sausages with substitution of pork fat by *Auricularia cornea* (AC).

| Parameters       | Control  | AC25 | AC50 | AC75 | AC100 |
|------------------|----------|------|------|------|-------|
| Valine           | 1.61 ± 0.02e | 1.48 ± 0.05d | 1.43 ± 0.00c | 1.29 ± 0.01b | 0.96 ± 0.00a |
| Threonine        | 0.67 ± 0.00e | 0.64 ± 0.00d | 0.60 ± 0.00c | 0.54 ± 0.01b | 0.47 ± 0.02a |
| Lysine           | 1.41 ± 0.00d | 1.30 ± 0.06c | 1.30 ± 0.00c | 1.13 ± 0.00b | 0.95 ± 0.00a |
| Methionine       | 0.16 ± 0.00c | 0.15 ± 0.00c | 0.12 ± 0.01b | 0.08 ± 0.01a | 0.10 ± 0.00a |
| Isoleucine       | 0.53 ± 0.00a | 0.63 ± 0.01b | 0.70 ± 0.00c | 0.77 ± 0.01d | 0.79 ± 0.00b |
| Leucine          | 0.93 ± 0.01a | 1.09 ± 0.00b | 1.24 ± 0.03c | 1.31 ± 0.01d | 1.38 ± 0.03a |
| Phenylalanine    | 0.73 ± 0.01d | 0.73 ± 0.00d | 0.66 ± 0.00c | 0.60 ± 0.00b | 0.51 ± 0.03a |
| Histidine        | 0.58 ± 0.02d | 0.53 ± 0.00cd | 0.51 ± 0.06bc | 0.46 ± 0.01ab | 0.45 ± 0.00a |

Non-essential

| Parameters  | Control | AC25 | AC50 | AC75 | AC100 |
|-------------|---------|------|------|------|-------|
| Serine      | 0.71 ± 0.00e | 0.64 ± 0.00d | 0.58 ± 0.00c | 0.56 ± 0.01b | 0.48 ± 0.01a |
| Arginine    | 1.16 ± 0.04d | 1.03 ± 0.00c | 1.01 ± 0.03c | 0.89 ± 0.00b | 0.74 ± 0.00a |
| Glycine     | 0.92 ± 0.00e | 0.84 ± 0.00d | 0.67 ± 0.00c | 0.66 ± 0.00b | 0.56 ± 0.00a |
| Aspartic acid| 1.64 ± 0.00d | 1.62 ± 0.01d | 1.53 ± 0.03c | 1.40 ± 0.06b | 1.15 ± 0.05a |
| Glutamic acid| 2.65 ± 0.03d | 2.59 ± 0.05d | 2.40 ± 0.05c | 2.17 ± 0.03c | 1.87 ± 0.01a |
| Alanine     | 0.99 ± 0.00d | 0.91 ± 0.03c | 0.94 ± 0.03c | 0.77 ± 0.00b | 0.66 ± 0.00a |
| Proline     | 0.88 ± 0.03a | 0.91 ± 0.03a | 0.95 ± 0.00b | 1.17 ± 0.02c | 1.26 ± 0.26d |
| Cysteine    | 0.09 ± 0.00c | 0.08 ± 0.00b c | 0.07 ± 0.01b | 0.07 ± 0.00b | 0.04 ± 0.01a |
| Tyrosine    | 0.56 ± 0.01c | 0.49 ± 0.01c | 0.49 ± 0.00c | 0.42 ± 0.00b | 0.35 ± 0.00a |

Notes: Values are given as mean ± standard error. Different letters in the same row indicate significant differences (p < 0.05). Control (0%), AC25 (25%), AC50 (50%), AC75 (75%), AC100 (100%) substitution of pork fat by AC, respectively.

3.5. Textural Profile Analysis

TPA is an important indicator to evaluate the quality and acceptability of comminuted meat products, which is expressed as hardness, springiness, gumminess, chewiness, and cohesiveness [25]. The results of TPA of sausages is listed in Table 3, and significant differences (p < 0.05) were observed among the sausages. With the improve of AC content, the hardness, gumminess, and cohesiveness of sausages were decreased, but the springiness and chewiness were increased. Compared with the control, hardness, gumminess, and cohesiveness of replacement group decreased by 9.29%–33.91%, 36.25%–72.51%, and 29.09%–58.18%, indicating that these sausages had a softer texture. This downtrend was similar to low-fat burger with high beta-glucans and oat-hull-based ingredient as fat replacer [27]. By contrast, the springiness and chewiness of replacement group increased by 2.29%–20.86%, and 1.87%–8.09% in comparison with the control, suggesting that these sausages required more energy to be compressed and the work during chewing will be slightly greater. It could be attributed to the highest amount of AC. The moisture and fat content also could influence TPA parameters [38]. These results may be attributed to the fiber and the rich and elastic properties of AC. This result complies with the report by dos Santos Alves [31] where animal fat was replaced with pork skin-based emulsion gels. Similar observations were also made by [11] who reported that using *Pleurotus eryngii* as replacements for pork back fat in sausages.

3.6. Free Amino Acids

Amino acids are important for the nutritional value and sensory properties of sausages [4]. The amino acids of sausages formulated with AC replaced pork fat are shown in Table 4. The main essential amino acids in the control group were valine, lysine, and leucine, and the main non-essential amino acids were glutamate, aspartic, and arginine. By contrast, the main essential amino acids in the replacement group were leucine, valine, and lysine,
and the main non-essential amino acids were glutamate, proline, and aspartate. Compared with the control group, the contents of isoleucine, leucine, and proline in the replacement group increased by 18.87%–49.06%, 17.20%–48.39%, and 3.41%–43.18%, respectively. It may be due to the AC being abundant in these amino acids. Amino acids were linked with multiple health benefits. In the literature, isoleucine was a potent plasma glucose-lowering amino acid [32]. Zhang and Guo et al. [33] found that increasing dietary leucine intake could reduce diet-induced obesity and improve glucose and cholesterol metabolism in mice via multi mechanisms. Proline is critically essential for nutrition, antioxidative reactions, and immune responses [34]. In addition, the control was higher than other amino acids of replacement group. These results could be explained by the fact that the difference of amino acid content between AC and pork fat.

3.7. Free Fatty Acids

The results of free fatty acids in sausages with substitution of pork fat with AC are listed in Table 5. Significant differences (p < 0.05) were observed among the samples. The highest content of fatty acids in all the samples was oleic acid, followed by palmitic acid, stearic, and linoleic acid, indicating that replacement of pork back fat by AC did not change the major fatty acids. This aspect was similar to the previous study of [32] in Toscana sausage. With the increase of AC content, palmitic acid, palmitoleic acid, oleic acid, and arachidonic acid increased by 0.72%–5.10%, 10.71%–41.67%, 1.11%–20.04%, and 2.44%–75.61% in comparison with the control, respectively. It may be due to the AC being rich in these fatty acids. The palmitoleic acid could improve hyperglycemia and hypertriglyceridemia by increasing insulin sensitivity [35] showed that oleic acid plays a role in immunomodulation, and in treating and preventing cardiovascular or autoimmune diseases, metabolic disturbances, skin injury, and cancer. Arachidonic acid plays an important role in the brain, cognitive functions, skeletal muscle, and immune systems, which could also promotes and regulates type 2 immune responses against intestinal [36]. However, the control was higher than other fatty acids in the replacement group.

### Table 5. Free fatty acids of sausages with substitution of pork fat by *Auricularia cornea* (AC).

| Fatty Acid | Control | AC25 | AC50 | AC75 | AC100 |
|------------|---------|------|------|------|-------|
| (C12:0)    | 0.08 ± 0.00 b | 0.08 ± 0.00 b | 0.08 ± 0.00 b | 0.07 ± 0.00 a,b | 0.06 ± 0.00 a |
| (C14:0)    | 1.37 ± 0.02 d | 1.31 ± 0.01 c | 1.26 ± 0.01 b | 1.25 ± 0.00 b | 1.23 ± 0.00 a |
| (C16:0)    | 23.72 ± 0.00 a | 23.89 ± 0.01 b | 24.15 ± 0.00 c | 24.31 ± 0.00 d | 24.93 ± 0.00 e |
| (C17:0)    | 0.32 ± 0.00 d | 0.27 ± 0.00 c | 0.28 ± 0.00 c | 0.24 ± 0.01 b | 0.20 ± 0.01 a |
| (C18:0)    | 15.73 ± 0.00 e | 15.16 ± 0.00 d | 14.69 ± 0.01 c | 14.49 ± 0.00 b | 11.81 ± 0.01 a |
| (C20:0)    | 0.26 ± 0.01 d | 0.24 ± 0.00 c | 0.23 ± 0.01 b,c | 0.22 ± 0.00 b | 0.16 ± 0.00 a |
| SFA        | 41.48 ± 0.01 e | 40.95 ± 0.02 d | 40.69 ± 0.01 c | 40.58 ± 0.01 b | 38.39 ± 0.02 a |
| (C18:1)    | 38.78 ± 0.00 a | 39.21 ± 0.01 b | 39.46 ± 0.01 c | 42.43 ± 0.00 d | 46.55 ± 0.00 e |
| (C16:1)    | 1.68 ± 0.01 a | 1.86 ± 0.00 b | 1.90 ± 0.01 c | 2.31 ± 0.00 d | 2.38 ± 0.00 e |
| (C17:1)    | 0.25 ± 0.01 d | 0.23 ± 0.01 c | 0.23 ± 0.00 c | 0.17 ± 0.00 b | 0.15 ± 0.01 a |
| (C20:1)    | 1.71 ± 0.01 c | 1.46 ± 0.01 b | 1.46 ± 0.00 b | 1.47 ± 0.00 b | 0.92 ± 0.04 a |
| MUFA       | 42.42 ± 0.03 a | 42.76 ± 0.03 b | 43.05 ± 0.02 c | 46.38 ± 0.00 d | 50.00 ± 0.04 e |
| (C18:2)    | 15.60 ± 0.01 e | 15.48 ± 0.01 d | 14.39 ± 0.00 c | 12.15 ± 0.00 b | 9.76 ± 0.00 a |
| (C20:4)    | 0.41 ± 0.00 a | 0.42 ± 0.01 a | 0.47 ± 0.01 b | 0.66 ± 0.00 c | 0.72 ± 0.00 d |
| PUFA       | 16.01 ± 0.01 e | 15.90 ± 0.00 d | 14.86 ± 0.01 c | 12.81 ± 0.00 b | 10.48 ± 0.00 a |

Notes: Values are given as mean ± standard error. Different letters in the same row indicate significant differences (p < 0.05). Control (0%), AC25 (25%), AC50 (50%), AC75 (75%), AC100 (100%) substitution of pork fat by AC, respectively. SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

Excessive intake of Saturated fatty acid (SFA) will lead to the increase of fat content and cholesterol content in the body, which may be aggravate the risk of cardiovascular and cerebrovascular diseases [2]. The sausages incorporated with AC as a fat replacer showed less (1.28%–7.45%) SFA than the control. Additionally, Monounsaturated fatty acid (MUFA) of the replacement group increased by 2.44%–75.61% in comparison with the
control. In general, AC is a healthier food that could reduce fat intake and be beneficial to human health.

3.8. Sensory Evaluation

The results of sensory evaluation for the replacement of pork fat with AC in sausages are shown in Figure 1. Replacing pork fat with 25% AC in sausages caused no significant \((p > 0.05)\) change in sensory characteristics, indicating that a small amount of AC had no obvious effect on the sausage. With regard to appearance, odor, and taste, AC50 and AC75 showed a higher score, compared to the control. It may be due to AC having a unique flavor and reducing the greasy taste of sausages, which was very popular among the sensory evaluation team. This result is consistent with the report by [11] that the addition of fried Pleurotus eryngii as a substitution for pork back fat-enriched these free amino acids and improved the taste and flavor properties of sausages. By contrast, AC100 got the lowest score in appearance, odor, and taste. Additionally, texture scores of replacement group were decreased significantly with the increase of AC, because AC made the sausage a soft texture. That may be characteristic of AC is different from pork fat. These results were in line with the TPA observed by instrumental measurements (Table 4). The highest score of overall acceptability in all the samples was AC75, followed by AC50, AC25, control, and AC100, indicating that excessive AC would reduce the sensory quality of the sausage. In general, all sausage groups were judged as acceptable (Overall acceptability > 7), and the best one was the replacement of pork fat with 75% AC.

4. Conclusions

The substitution of pork fat with Auricularia cornea (AC) in sausages was found to be a viable alternative in this study. AC improved the protein, moisture, and ash contents, especially isoleucine, leucine, proline, palmitic, palmitoleic, oleic, and arachidonic acids in the sausages. In addition, cooking loss and water holding capacity were also improved in comparison with the control. Meanwhile, AC significantly reduced the fat (12.61%–87.56%) and energy (5.76%–56.40%) levels of the sausages. Furthermore, the sausages demonstrated the yellowness, whiteness, lightness, and soft texture on account of AC. From the sensory opinion, the best sausage formulation was 75% of pork fat replacement with AC. The results shown that AC could be possibility used as healthier food to decrease the content of pork fat in sausages.

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References
1. Robert, P.; Zamorano, M.; González, E.; Silva-Weiss, A.; Cofrades, S.; Giménez, B. Double emulsions with olive leaves extract as fat replacers in meat systems with high oxidative stability. Food Res. Int. 2019, 120, 904–912. [CrossRef]
2. Kwon, H.C.; Shin, D.M.; Yune, J.H.; Jeong, C.H.; Han, S.G. Evaluation of gels formulated with whey proteins and sodium dodecyl sulfate as a fat replacer in low-fat sausage. Food Chem. 2020, 337, 127682. [CrossRef] [PubMed]
3. de Souza Paglarini, C.; de Figueiredo Furtado, G.; Honorio, A.R.; Mokarzel, L.; da Silva Vidal, V.A.; Ribeiro, A.P.B.; Pollomio, M.A.R. Functional emulsion gels as pork back fat replacers in Bologna sausage. Food Struct. 2019, 20, 100105. [CrossRef]
4. Wang, X.; Xie, Y.; Li, X.; Liu, Y.; Yan, W. Effects of partial replacement of pork back fat by a camellia oil gel on certain quality characteristics of a cooked style Harbin sausage. *Meat Sci.* 2018, 146, 154–159. [CrossRef] [PubMed]

5. Kılıç, B.; Özer, C.O. Potential use of interesterified palm kernel oil to replace animal fat in frankfurters. *Meat Sci.* 2019, 148, 206–212. [CrossRef] [PubMed]

6. Oh, I.; Lee, J.; Lee, H.G.; Lee, S. Feasibility of hydroxypropyl methylcellulose oelgel as an animal fat replacer for meat patties. *Food Res. Int.* 2019, 122, 566–572. [CrossRef]

7. Saygi, D.; Erçokşun, H.; Sahin, E. Hazelnut as functional food component and fat replacer in fermented sausage. *J. Food Sci. Technol.* 2018, 55, 3385–3390. [CrossRef]

8. Utama, D.T.; Jeong, H.S.; Kim, J.; Barido, F.H.; Lee, S.K. Fatty acid composition and quality properties of chicken sausage formulated with pre-emulsified perilla-canaola oil as an animal fat replacer. *Poult. Sci.* 2019, 98, 3059–3066. [CrossRef]

9. de Carvalho, F.A.L.; Munekata, P.E.; Pateiro, M.; Campagnol, P.C.; Domínguez, R.; Trindade, M.A.; Lorenzo, J.M. Impact of salt reduction on the nutritional content and health characteristics of a cooked style Harbin sausage. *Meat Sci.* 2018, 146, 154–159. [CrossRef] [PubMed]

10. Wang, L.; Guo, H.; Liu, X.; Jiang, G.; Li, C.; Li, X.; Li, Y. Roles of partial replacement of pork back fat by a camellia oil gel on certain quality properties of chicken sausage as the pork lean meat replacer in production of Lentinula edodes fruiting body extract in H22 bearing mice. *Food Chem.* 2017, 231, 33–41. [CrossRef] [PubMed]

11. Liu, X.; Zhang, K. A review of the advances of the research on a white variant strain in Genus *Auricularia*. *Edible Med. Mushrooms* 2016, 24, 230–233. (In Chinese)

12. Si, F.; Liu, X.; Deng, J. Optimization of extraction process and physicochemical properties of dietary fiber from *Auricularia cornea* var. *Li* Root. *Food Ferment. Ind.* 2019, 45, 209–214. [CrossRef] [PubMed]

13. Cao, Y.; Bao, H.; Li, X.; Bao, T.; Li, Y. Anti-tumor activities of *Auricularia cornea* fruiting body extract in H22 bearing mice. *Mycosystema* 2017, 36, 1289–1298. (In Chinese)

14. Guedes-Oliveira, J.M.; Costa-Lima, B.R.C.; Oliveira, D.; Neto, A.; Deliza, R.; Conte-Junior, C.A.; Guimarães, C.F.M. Mixture design approach for the development of reduced fat lamb patties with carboxymethyl cellulose and inulin. *Food Sci. Nutr.* 2019, 7, 1328–1336. [CrossRef] [PubMed]

15. Liu, X.; Qu, H.; Gou, M.; Guo, H.; Wang, L. Effects of Pine Bark Extract on Physicochemical Properties and Biological Activity of Active Chitosan Film by Bionic Structure of Dragonfly Wing. *Coatings* 2021, 11, 1077. [CrossRef]

16. Jridi, M.; Abdelhedi, O.; Souissi, N.; Kammoun, M.; Nasri, M.; Ayadi, M.A. Improvement of the physicochemical, textural and microbiological approach. *Food Chem.* 2020, 340, 128095. [CrossRef] [PubMed]

17. Liu, X.; Cong, M.; Teng, X.; Peng, M.; Ren, L.; Wang, L. Effects of Pine Bark Extract on Physicochemical Properties and Biological Activity of Active Chitosan Film by Bionic Structure of Dragonfly Wing. *Coatings* 2021, 11, 1077. [CrossRef]

18. Liu, X.; Qu, H.; Gou, M.; Guo, H.; Wang, L.; Yan, X. Application of *Weissella cibaria* X31 or *Weissella confusa* L2 as a starter in low nitrite dry-fermented sausages. *Int. J. Food Eng.* 2020, 16, 20190344. [CrossRef]

19. Wang, L.; Guo, H.; Liu, X.; Jiang, G.; Li, C.; Li, X.; Li, Y. Roles of *Lentinula edodes* as the pork lean meat replacer in production of the sausage. *Meat Sci.* 2019, 156, 44–51. [CrossRef]

20. Chen, Y.; Jia, X.; Sun, F.; Jiang, S.; Liu, H.; Liu, Q.; Kong, B. Using a stable pre-emulsified canola oil system that includes porcine plasma protein hydrolysates and oxidized tannic acid to partially replace pork fat in frankfurters. *Meat Sci.* 2020, 160, 107968. [CrossRef]

21. Monteiro, G.; Souza, X.; Costa, D.; Faria, P.; Vicente, J. Partial substitution of pork fat with canola oil in Toscana sausage. *Innov. Food Sci. Emerg. Technol.* 2017, 44, 2–8. [CrossRef]

22. Summo, C.; De Angelis, D.; Difonzo, G.; Caponio, F.; Pasqualone, A. Effectiveness of oat-hull-based ingredient as fat replacer to produce low nitrite dry fermented sausages. *Int. J. Food Eng.* 2020, 16, 20190344. [CrossRef]

23. Los Santos, M.; Munekata, P.E.; Pateiro, M.; Magalhães, G.C.; Barreto, A.C.S.; Lorenzo, J.M.; Pollonio, M.A.R. Pork skin-based emulsion gels as animal fat replacements in hot-dog style sausages. *LWT* 2020, 132, 109845. [CrossRef]

24. Sousa, S.C.; Fragoso, S.P.; Penna, C.A.R.; Araújo, N.M.O.; Silva, F.A.P.; Ferreira, V.C.S.; Araújo, I.B.S. Quality parameters of frankfurter-type sausages with partial replacement of fat by hydrolyzed collagen. *LWT Food Sci. Technol.* 2017, 76, 320–325. [CrossRef] [PubMed]

25. Salcedo-Sandovall, L.; Cofrades, S.; Ruiz-Capillas, C.; Sola, M.T.; Jiménez-Colmenero, F. Healthier oils stabilized in konjak matrix as fat replacers in n-3 PUFA enriched frankfurters. *Meat Sci.* 2013, 93, 757–766. [CrossRef] [PubMed]
31. dos Santos Alves, L.A.A.; Lorenzo, J.M.; Gonçalves, C.A.A.; Dos Santos, B.A.; Heck, R.T.; Cichoski, A.J.; Campagnol, P.C.B. Production of healthier bologna type sausages using pork skin and green banana flour as a fat replacers. *Meat Sci.* 2016, 121, 73–78. [CrossRef] [PubMed]

32. Doi, M.; Yamaoka, I.; Fukunaga, T.; Nakayama, M. Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. *Biochem. Biophys. Res. Commun.* 2003, 312, 1111–1117. [CrossRef] [PubMed]

33. Zhang, Y.; Guo, K.; LeBlanc, R.E.; Loh, D.; Schwartz, G.J.; Yu, Y.H. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. *Diabetes* 2007, 56, 1647–1654. [CrossRef] [PubMed]

34. Wu, G.; Bazer, F.W.; Burghardt, R.C.; Johnson, G.A.; Kim, S.W.; Knabe, D.A.; Spencer, T.E. Proline and hydroxyproline metabolism: Implications for animal and human nutrition. *Amino Acids* 2011, 40, 1053–1063. [CrossRef] [PubMed]

35. Yang, Z.H.; Miyahara, H.; Hatanaka, A. Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-A y Mice with genetic type 2 diabetes. *Lipids Health Dis.* 2011, 10, 120. [CrossRef] [PubMed]

36. Tallima, H.; El Ridi, R. Arachidonic acid: Physiological roles and potential health benefits—A review. *J. Adv. Res.* 2018, 11, 33–41. [CrossRef] [PubMed]