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Debaryomyces hansenii Strains from Traditional Chinese Dry-Cured Ham as Good Aroma Enhancers in Fermented Sausage

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Abstract: In some countries, yeasts are still not allowed in the production of commercially fermented sausages. Therefore, further research is needed on producing fermented meat products using different strains of yeasts. In this study, two strains of *Debaryomyces hansenii* (*D. hansenii* Y61 and Y67) were inoculated in fermented sausages to study their effects as starter cultures. The inoculation of *D. hansenii* strains affected ripening by decreasing the pH and a_w. The sausages inoculated with Y61 and Y67 exhibited decreases in lipid oxidation of 40.70% and 36.04%, respectively, and *Enterobacteriaceae* counts of 50% and 100%, respectively. The inoculating yeasts Y61 and Y67 increased the lightness (L*) and redness (a*) of fermented sausages. The *D. hansenii*-inoculated sausages had higher levels of free amino acids and fatty acids, which improved the digestibility, sensory value, and safety of these sausages. Moreover, the total amount of ester compounds increased by 87.14% and 83.31% in the Y61- and Y67-inoculated groups, respectively, which contributed to the aroma. Better sensory attributes were also found in the sausages inoculated with Y61 and Y67 *D. hansenii*. Native *D. hansenii* Y61 and Y67 are, therefore, good starter cultures for fermented sausage production. Together, the results provide data supporting future research and the use of yeast-fermented sausages.

Keywords: yeast; fermented meat product; flavor; volatile compound

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1. Introduction

Fermented meat products are mainly produced in the Mediterranean area in Spain, France, Italy, etc., but also in other parts of the world, such as China and the USA, where they are an important part of people's diet [1,2]. In China, the primary types of fermented meat products include dry-cured ham (such as Xuanwei, Rugao, and Jinhua hams), fermented sausage (such as Sichuan and Cantonese sausage), and fermented loin. The typical fermented meat products use specific microorganisms to complete the fermentation process [3,4]. Because of the role of microorganisms such as bacteria, yeasts, and molds, carbon and nitrogen sources are transformed into various organic acids, alcohols, and some bioactive substances, which result in unique appearances; intense cured flavors; ancient, traditional tastes; and longer shelf lives [5–8].

Traditional meat products are usually naturally fermented, but this process may lead to uncertainty in the dominant microflora, which makes it difficult to guarantee the quality of products. In recent years, researchers have focused on functional starters for the fermentation of meat products because these starters ensure the product is high-quality, nutritious, and has a good taste [9–12]. Therefore, an increasing number of autochthonous microorganisms are screened from traditional dry-cured meat products for dry and semi-dry meat production to improve their quality [13,14].

Lactic acid bacteria, *Staphylococcus*, yeasts, and molds are the main microorganisms used in the fermentation process of meat products [4]. During natural fermentation, yeast up to 10^6-10^7 cfu/g yeast is usually found in fermented meat products such as fermented sausage [4]. Such high numbers of yeast indicate that the yeast microflora could play an important role in the maturation process, and this could be one of the most beneficial microflorae in fermented meat products. Since the 1970s, scientists, especially from Europe, have carried out extensive research into the important effects of yeast as starter cultures in the manufacture of meat products [15]. During meat processing, through aerobic fermentation, yeast gradually depletes the residual oxygen in meat products, which is conducive to the stability of color, thus giving these products a bright color [16]. Moreover, some of the most common members of the microflora in fermented meat products are yeasts. They provide unique flavors caused by decomposing lipids and proteins. This process produces aldehydes, alcohols, esters, and other small molecular compounds, resulting in a unique flavor [15]. Autochthonous yeast from dry-cured meat products contribute to aroma development by increasing the perception of fruity and cured aroma notes due to their ability to generate sulfur and ester compounds [17]. Yeasts can also be used as probiotics to play a vital role as biocontrol agents against pathogenic microorganisms [10]. Moreover, yeasts are relatively safe organisms for producing fermented meat because they seldom produce toxins that are harmful to humans. Because of their nontoxic properties, yeasts are one of the biological agents recommended by the Qualified Presumption of Safety List [18]. Based on the previous reports, the main strains isolated from dry-cured meat products belong to the species *Debaryomyces hansenii* (*D. hansenii*), *Candida zeylanoides*, *Debaryomyces maramus*, and, to a lesser extent, *Candida famata* and *Hyphopichia burtonii*, among which *D. hansenii* is the predominant species [19,20]. In addition, the potential probiotic beneficial effects of *D. hansenii* have been reported by Ochangco et al. [21].

Although some scientists have suggested that the influence of yeasts on the ripening of fermented sausages is doubtful [22], information about using native yeasts as starter cultures for manufacturing traditional Chinese fermented meat products is rare. Unlike many European countries, in China, yeast is still not allowed in the production of commercially fermented sausages. Therefore, further research is needed regarding the production of fermented meat products using different strains of yeast, especially regarding enhanced products. In our previous research, based on the results of culture media studies, two *D. hansenii* strains isolated from traditional dry-cured ham (Xuanwei ham), Y61 and Y67, showed a remarkable aroma-enhancing ability. They also showed good fermenting characteristics, including salt tolerance, nitrite tolerance, acid production, low-temperature tolerance, and high activity levels of lipase and protease (unpublished data). It was therefore suggested that they could be good starter cultures for fermented meat production. Based on these results, to determine the role of yeast as good aroma enhancers in fermented sausages, two *D. hansenii* strains were used as inoculating starters for fermented sausage production, and the quality of the fermented sausages, including physio-chemical parameters, flavor, sensory properties, oxidation degree, proteins, and lipid decomposition, were determined. These results added to the fermented meat product starter culture bank data and provided more information about the use of autochthonous yeast from Chinese traditional meat products in manufacturing fermented sausages.

2. Materials and Methods

2.1. Preparation of a Yeast Strain Starter

Two strains of *D. hansenii*, Y61 and Y67, were identified by screening traditional dry-cured Xuanwei ham. These strains were selected for their aromatic potential to be used as starters in sausage fermentation. The yeasts were cultivated on YPD medium until reaching a concentration of 10^7 cfu/mL. Subsequently, they were separated by centrifugation at 7000 rpm for 10 min at 4 °C. The collected yeast powders were then vacuum freeze-dried overnight with a protective agent consisting of 16.0% skim milk, 10.0% trehalose, 2.0% vitamin C, and 2.0% glycerol. The ratio of protective agent to the cell suspension was

2:1. The resulting freeze-dried yeast powder was stored at −80 °C until it was needed for further experiments.

2.2. Preparation of Dry-Cured Fermented Sausages

There were three batches: the control batch (without yeast), the Y61 batch (inoculated with Y61 yeast strain), and the Y67 batch (inoculated with Y67 yeast strain). Each batch consisted of twenty-two sausages. The sausages were made using lean pork (75%) and back fat (25%), along with the following additives (g/kg): NaCl (28), sugar (10), liquor (15), chili powder (8), Sichuan pepper powder (5), black pepper powder (0.5), five-spice powder (star anise, clove, cinnamon, fennel, and brown pepper, 0.5), and sodium nitrite (0.075). The meat was ground and vacuum-minced with the ingredients, then marinated for 10 h at 4 °C. After marination, the meat was inoculated with starter culture yeasts Y61 or Y67 at a concentration of 2% and a concentration of 10^7 cfu/mL. The mixture was then stuffed into pig casings at 4 °C. The diameter of the sausage casings was about 2 cm, and each sausage weighed about 50 g. Based on calculations, the initial yeast count of the sausage was approximately 10^7 cfu.

All sausage batches were ripened in the same drying chamber, undergoing an initial stage at 25–30 °C and 80–90% relative humidity for 3 days, followed by drying at 10 °C and 70–75% HR for 15 days. During the ripening process, the weight losses and pH values were measured.

2.3. Chemical Analysis

The pH of the sausages was measured using a pH meter (FE20; Mettler-Toledo Instruments, Shanghai, China). The water activity (a_w) of sausages was measured using an FA-st/1 apparatus by GBX (FA-st; GBX, FR). The lipid oxidation of the meat was measured using the fluorescence 2-thiobarbituric reactive substances (TBARS) method. The results are expressed as mg malonaldehyde (MDA)/kg of dry matter.

Microbial analysis was conducted to determine the total number of bacteria, yeasts, and *Enterobacteriaceae*. The minced sausage samples were homogenized with sterile saline solution for 1 min, and then 0.1 mL of diluted suspension was spread onto the surface of an appropriate agar medium. Yeasts were incubated at 28 °C for 72 h using Rose Bengal Agar with chloramphenicol. The total number of bacteria was determined using Standard I Nutrient Agar (g/L): peptone 15; yeast powder 3; NaCl 6; glucose 1; agar 12, and incubating at 37 °C for 48 h. *Enterobacteriaceae* were counted after incubation in Violet Red Bile Glucose Agar at 37 °C for 48 h.

Measurements of the color of the sausages were conducted on the sample surface using a LabScan colorimeter (LSXE; HunterLab, Reston, VA, USA), which was calibrated against black and white reference tiles. The CIE L* (lightness), a* (redness), and b* (yellowness) values were obtained using an illuminant A (light source). We used the average of two random readings from the top and bottom locations of samples for statistical analysis.

2.4. Texture Parameters

The texture of the samples was evaluated using a texture analyzer (TAXTplusC; Stable Micro System, Godalming, UK). The sausages were compressed to 40% of their original lengths using a cylindrical probe with a test speed of 1 mm/s. The values of adhesiveness, springiness, hardness, cohesiveness, and chewiness were directly obtained from the force–time curve.

2.5. Free Fatty Acids (FFAs)

The FFA composition was determined using chromatography/mass spectrometry (7890A-5975C; Agilent Technologies, San Jose, CA, USA) equipped with an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies). The analytical conditions of gas chromatography were as follows: the injector temperature was maintained at 250 °C, the detector temperature was maintained at 230 °C, and the carrier gas was helium flowing at a

rate of 1 mL/min. The split ratio was 10:1. The oven temperature program started at 170 °C for 2 min, then increased to 250 °C at 4 °C/min, and finally increased to 280 °C at a rate of 30 °C/min. The total run time was 26 min. Mass spectrometry was operated in the electron impact mode with an electron energy of 70 eV and a scan range from 50 to 550 amu. The FFAs were identified using retention time and peak areas from digital libraries (PBM/NIST, 2011), and the results are expressed as the percentage of the total fatty acid methyl esters.

2.6. Free Amino Acid

The 0.3 g sample was placed in a 10 mL volumetric bottle, and 8 mL of 0.02 M HCL was added. After performing solid-phase extraction column chromatography using a C_{18} column, 100 μL of clear liquid was separated and dried in a vacuum drying oven. Subsequently, 10 mL of 0.02 M HCL was added, and the mixture was vigorously shaken. A total of 500 μL of sample was separated and combined with 250 μL of 0.1 M phenyl isothiocyanate acetonitrile, as well as 250 μL of 1 M triethylamine acetonitrile, and then the sample was protected with nitrogen for 1 h, followed by analysis of a 0.45 μm organic film using an L-8800 amino acid analyzer (Hitachi, Tokyo, Japan).

2.7. Volatile Compounds

The small pieces of sausage weighing 5.0 g were placed in a 20 mL headspace vial. The flavor compounds were measured using gas chromatography/mass spectrometry (GC/MS) (7890A-5975C; Agilent Technologies) and an HP-5 silica capillary column (60 m × 0.32 mm × 0.25 μm; Agilent Technologies). The volatile compounds were extracted using headspace solid-phase microextraction (HS-SPME; Agilent Technologies). Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The SPME fiber was maintained in the injection port at 280 °C throughout the whole chromatographic run. The injection port was operated in splitless mode. The temperature was held isothermal at 35 °C for 1 min, then increased to 140 °C at a rate of 4 °C/min and maintained for 5 min. The transfer line to the mass spectrometer was maintained at 230 °C. The mass spectra were obtained by electronic impact at 70 eV. The data were collected at a rate of s^{-1} over a range of 33−450 to obtain the m/z values. The results are expressed as a percentage of the total chromatographic area.

2.8. Sensory Evaluation

The fermented sausage was washed and steamed for 20 min before being served on a plate. For sensory evaluations, we followed the methods of Yingying Hu et al. [23]. with some necessary adjustments. A panel of 20 members, including 10 females and 10 males who had received professional training, assessed the sensory properties of the sausages. These properties included color, smell, taste, sourness, chewiness, and overall acceptability. We used a 9-point hedonic scale for the evaluations.

2.9. Statistical Analysis

The effects of inoculation with different yeast strains on the chemical, microbial, and volatile compounds of sausages were tested using a two-factor analysis of variance (ANOVA). XLSTAT 2009.4.03 statistical software (Addinsoft, Barcelona, Spain) was used for the analysis. Differences between the means of the samples were analyzed using Fisher's Least Significant Difference test. Significant differences between two groups are indicated by different letters and determined at the $p < 0.05$ alpha level.

3. Results and Discussion

3.1. Physicochemical Analysis

It is widely accepted that changes in water content during sausage fermentation significantly impact the quality of sausages. Studies have shown that if the water content of fermented sausages is not rapidly reduced within 48 h, spoilage microorganisms will quickly breed and produce an off-odor [1]. During fermentation, the water content of sausages is related to the speed of surface evaporation. Due to the action of microorganisms,

the water activity of fermented meat products decreases rapidly, reducing the production cycle of the fermentation process and prolonging the storage time. This is why traditional fermented meat products are also called dry meat products [1]. Table 1 shows that the a_w of the yeast-inoculated groups (Y61 and Y67) was significantly lower than that of the control group. These results were inconsistent with a previous study, which reported no difference between yeast-inoculated and uninoculated batches [13]. This phenomenon can be attributed to the microorganisms that decompose macromolecules (proteins, lipids, and glycogen) to produce flavor substances and other low-molecular-weight substances during fermentation. Degradation of these macromolecules reduces their binding water content, leading to a significant reduction in water activity of the yeast-inoculated groups. The pH also plays an important role in fermented sausages, as a low pH accelerates the drying speed [1]. Table 1 shows that the pH of the Y61 and Y67 strains was significantly lower than the control group, with final pH values of 4.76 and 4.92, respectively. These results differ from some reports on fermented sausages [7,24,25], which found no change or a rise in pH. Our previous results showed that Y61 and Y67 had good acid-producing abilities, and a large number of yeast proliferated (Table 1), decomposing organic matter to produce organic acids. This process may explain why the pH of yeast-fermented sausages was lower than that of naturally fermented sausages. Another reason may be that the lactic acid bacteria in both inoculation groups played a role in acid production. Under acidic conditions, the growth of pathogens and spoilage bacteria was inhibited, resulting in lower counts of total bacteria and *E. coli*, thus prolonging the sausage shelf life (Table 1).

Table 1. Effects of *D. hansenii* inoculation on physicochemical parameters, fluorescence 2-thiobarbituric reactive substances value, color, and microbial counts in fermented sausages ripening for 18 days.

Parameters	Group		
	Control	***D. hansenii* Y61**	***D. hansenii* Y67**
pH	5.96 ± 0.05^{a}	4.76 ± 0.08^{b}	4.92 ± 0.01^{b}
a_w	0.81 ± 0.002^{a}	0.76 ± 0.001^{b}	0.74 ± 0.001^{b}
TBARS (mg/kg)	0.88 ± 0.031^{a}	0.52 ± 0.020^{c}	0.55 ± 0.052^{b}
Brightness (L*)	35.44 ± 0.13^{b}	39.78 ± 0.62^{b}	41.14 ± 0.01^{a}
Redness (a*)	5.38 ± 0.04^{b}	13.88 ± 0.9^{a}	13.01 ± 0.01^{a}
Yellowness (b*)	12.25 ± 1.14^{c}	8.63 ± 0.06^{a}	9.23 ± 0.02^{b}
Yeasts (cfu/g)	$9.1 \times 10^{2\,c}$	$3.79 \times 10^{4\,b}$	$4.27 \times 10^{4\,a}$
Total bacteria (cfu/g)	$6.24 \times 10^{4\,a}$	$4.71 \times 10^{4\,c}$	$5.92 \times 10^{4\,b}$
Enterobacteriaceae (cfu/g)	2.00^{c}	1.00^{b}	0^{a}

The values are the mean ± standard deviation. On the same row, means with different letters significant differences ($p < 0.05$), $n = 5$.

For meat products, the main reason for declining quality is oxidative deterioration. This process of lipid oxidation can alter the flavor, taste, and texture of meat, leading to a loss of nutrients and, ultimately, a decrease in quality [26], even producing toxic substances [27]. Previous results showed that professional sensory evaluators could detect oxidized taste when the TBARS value is between 0.5 and 1.0 MDA/kg per sample, while ordinary consumers can detect it when the TBARS value is between 0.6 and 2.0 [26]. In our study, the TBARS values of different groups are presented in Table 1. For the Y61 and Y67 *D. hansenii* incubation groups, the TBARS values were 0.52 and 0.55, respectively, which were significantly lower ($p < 0.05$) than the control group of 40.70% and 36.04%, respectively. Similar results were reported in previous studies [5,24]. The lower levels of TBARS from yeast-inoculated groups may be because yeast has oxygen-scavenging ability, and additionally, yeast can produce various metabolites with antioxidant activity, such as reduced glutathione, butyrate, and folate, which can increase the antioxidative activity of sausages [28].

Meat color is an important indicator of meat quality, as it directly influences consumers' purchasing decisions. Previous studies have shown that various factors, such as heme

pigments, oxidation status, and physical characteristics (pH, temperature, and storage times), can affect meat color [29]. As shown in Table 1, the inoculation of Y61 and Y67 yeast increased lightness (L*) and redness (a*) and decreased yellowness (b*) of fermented sausages. During meat processing, yeast gradually depletes the residual oxygen in meat products through aerobic fermentation, which contributes to color stability and gives the products a vibrant color. Consequently, the yeast-inoculated groups exhibited better color stability. One study [30] reported that lipid oxidation leads to myoglobin oxidation, resulting in a gray color of meat products. The higher TBARS value in the control group could thus partly explain the higher b* value observed.

According to this study, the number of yeast in yeast-inoculated groups reached 3.79×10^4 (Y61 batch) and 4.27×10^4 cfu/g (Y67 batch), which was significantly higher than that of the control group (9.1×10^2 cfu/g). Furthermore, the total bacteria count and the number of *Enterobacteriaceae* in the two inoculation groups were significantly lower than that in the control group. This phenomenon can be attributed to the yeast becoming the dominant microorganism in the fermented sausages, inhibiting the growth of other bacteria during processing. Additionally, the action of yeast can lead to the production of certain small molecules, such as organic acids, which inhibit the growth of harmful bacteria [21].

3.2. Texture

Texture is a key factor for consumers when evaluating the quality and freshness of food [31]. Hardness, springiness, cohesiveness, gumminess, and chewiness are all indicators of the texture quality of food. It is commonly believed that the greater the chewiness, the better the taste. Additionally, there is a positive correlation between the cohesiveness and the cell binding force, so the higher the cohesiveness, the more tender the meat products [31].

As shown in Table 2, the values of hardness, cohesiveness, gumminess, and chewiness were significantly higher in the fermented sausages inoculated with Y61 and Y67 *D. hansenii* compared to the control group. However, no difference in springiness was observed. These findings align with a study conducted by Ramos-Moreno et al. [7]. This phenomenon could be attributed to the use of *D. hansenii* yeast as a fermentation starter culture. This yeast promotes water loss and decreases the pH value, leading to closer muscle fiber arrangement and increased chewiness. Additionally, yeast can facilitate cross-linking between protein molecules, resulting in greater cohesiveness and gumminess. These results confirm the role of *D. hansenii* yeast in water release and subsequent drying during the processing of fermented sausage. Overall, the Y61 group demonstrated a stronger effect in improving the texture of fermented sausage compared to the Y67 group.

Table 2. Texture parameters of different batches of sausages ripening for 18 days.

Parameters	Group		
	Control	***D. hansenii* Y61**	***D. hansenii* Y67**
Hardness/(N·cm^{-2})	1493.0 ± 21.22 ^{c}	7577.7 ± 37.56 ^{a}	4245.0 ± 31.58 ^{b}
Springiness/cm	0.76 ± 0.01 ^{a}	0.70 ± 0.05 ^{a}	0.71 ± 0.01 ^{a}
Cohesiveness	0.59 ± 0.02 ^{c}	0.72 ± 0.05 ^{a}	0.67 ± 0.02 ^{b}
Gumminess/(N·S)	1080.6 ± 37.66 ^{c}	4684.21 ± 380.77 ^{a}	2557.26 ± 105.32 ^{b}
Chewiness/(N·cm^{-1})	825.10 ± 24.38 ^{c}	3313.25 ± 489.24 ^{a}	1805.78 ± 108.73 ^{b}

The values are the mean ± standard deviation. On the same row, means with different letters differ significantly ($p < 0.05$), $n = 5$.

3.3. Free Amino Acids

From a nutritional perspective, amino acids are divided into three categories: essential amino acids (EAA), semi-essential amino acids, and conditionally essential amino acids. The EAAs are required by the human body because they cannot be synthesized or synthesized at a rate that meets the needs of the body. Therefore, EAAs are indispensable when compared to the other two types of amino acids. Table 3 shows that different batches of

fermented sausages contained 17 types of FAAs, out of which 6 were EAAs (tryptophan could not be detected using the method). Among the different batches, arginine (Arg) comprised the highest content. The total content of FAAs in the two yeast-inoculated groups was higher than that in the control group, with an increase of 11.97% (8785.06 mg/kg) and 20.19% (9244.18 mg/kg), respectively. In terms of EAAs, compared to the control group, the total EAAs of the two yeast-inoculated groups were increased by 29.65% (1511.48 mg/kg) and 24.16% (1447.57 mg/kg), respectively.

Table 3. The free amino acid profile and content in different batches of sausages ripening for 18 days (mg/kg).

FAA	Group		
	Control	***D. hansenii* Y61**	***D. hansenii* Y67**
Asp	64.1 ± 0.15 ^{b}	60.38 ± 0.01 ^{c}	79.79 ± 0.02 ^{a}
Glu	487.07 ± 0.43 ^{c}	662.1 ± 0.50 ^{b}	714.0 ± 0.37 ^{a}
Cys	4.4 ± 0.02 ^{b}	13.33 ± 0.05 ^{a}	nd ^{c}
Ser	160.95 ± 0.01 ^{c}	193.46 ± 0.05 ^{b}	199.86 ± 0.03 ^{a}
Gly	220.07 ± 0.33 ^{b}	268.86 ± 0.37 ^{a}	265.35 ± 0.40 ^{a}
His	533.29 ± 0.15 ^{b}	268.86 ± 0.26 ^{c}	695.3 ± 0.30 ^{a}
Arg	3948.71 ± 0.42 ^{b}	4388.21 ± 0.56 ^{a}	4418.5 ± 0.31 ^{a}
Thr *	131.78 ± 0.25 ^{c}	151.85 ± 0.23 ^{b}	155.66 ± 0.21 ^{a}
Ala	502.6 ± 0.05 ^{c}	624.61 ± 0.02 ^{a}	606.01 ± 0.05 ^{b}
Pro	551.64 ± 0.04 ^{c}	600.28 ± 0.01 ^{b}	621.19 ± 0.05 ^{a}
Tyr	79.0 ± 0.02 ^{b}	111.95 ± 0.01 ^{a}	113.4 ± 0.02 ^{a}
Val *	169.59 ± 0.15 ^{b}	222.74 ± 0.21 ^{a}	219.66 ± 0.11 ^{a}
Met	128.48 ± 0.39 ^{a}	81.54 ± 0.22 ^{b}	83.2 ± 0.35 ^{b}
Lle *	128.48 ± 0.23 ^{b}	165.69 ± 0.17 ^{a}	163.81 ± 0.21 ^{a}
Leu *	202.91 ± 0.11 ^{b}	251.57 ± 0.15 ^{a}	249.21 ± 0.24 ^{a}
Phe *	103.47 ± 0.10 ^{b}	126.19 ± 0.03 ^{a}	123.81 ± 0.2 ^{a}
Lys *	429.63 ± 0.20 ^{c}	593.44 ± 0.20 ^{a}	535.42 ± 0.26 ^{b}
∑FAA	7846.17 ± 3.05 ^{c}	8785.06 ± 3.14 ^{b}	9244.18 ± 3.13 ^{a}
∑EAA	1165.86 ± 1.04 ^{c}	1511.48 ± 0.99 ^{a}	1447.57 ± 1.23 ^{b}

The values are the mean ± standard deviation. On the same row, means with different letters differed significantly ($p < 0.05$), $n = 5$; * = EAA: essential amino acid; nd: not detected.

During the fermentation of sausages, yeasts, particularly protease enzymes, can speed up the breakdown of proteins, resulting in higher levels of small peptides and free amino acids (FAAs). This can enhance the taste and flavor of sausages. It is widely recognized that FAAs, which are known as taste-active compounds, play a crucial role in imparting distinct flavors. The amount of FAAs directly affects the freshness of food [32,33]. FAAs can be categorized into several classes due to their nature and intensity of taste: usually, glycine (Gly), alanine (Ala), serine (Ser), threonine (Thr), proline (Pro), and hydroxyproline present a sweet taste; some basic and aliphatic amino acids such as isoleucine (Iso), leucine (Leu), arginine (Arg), histidine (His), lysine (Lys), phenylalanine (Phe), and valine (Val) present a bitter taste; and amino acids containing a sulfur atom (cysteine (Cys) and methionine (Met)) present a sulfuric note [32,33]. Some amino acids exhibit multiple tastes. For instance, Arg has a bitter taste with a hint of sweetness. Ser has a sweet taste combined with a sour and umami flavor, glutamine (Glu) offers a combination of sour and umami tastes, and Ala can present both sweetness and slight umami flavors [32,33]. Apart from the absolute quantity of FAAs, the relative balance between various FAAs also influences the overall taste of meat [32].

As shown in Table 3, the *D. hansenii* Y67 group exhibited a significant increase in the contents of Asp and Glu, with a 24.48% and 46.59% increase, respectively; this boost in Asp and Glu contributed to the enhanced umami taste of the fermented sausage. Additionally, the Y67 group showed increased levels of Gly, Ala, and Tyr, with a 20.57%, 20.57%, and 43.54% increase. These elevated levels of Gly, Ala, and Tyr resulted in a sweeter taste profile

for the fermented sausages in the Y67 group. Furthermore, the Y67 group demonstrated an increase in the contents of Gly, Ala, and Tyr by 22.17%, 24.28%, and 41.71%, respectively, further enhancing the sweetness of the fermented sausages in this group. In contrast, the Y61 group displayed a 35.93% increase in Glu content, which enhancing the umami taste of the fermented sausages when compared to the control group. Moreover, the Y61 group exhibited a 49.58% reduction in the content of His, resulting in a decrease in the bitter substances present in the fermented sausage. Overall, this study highlights the stronger improvement in good-taste substances observed in the Y67 group compared to the Y61 group.

3.4. Free Fatty Acids

Table 4 shows that 15 different types of free amino acids (FAAs) were detected in different batches of fermented sausages, including six types of saturated fatty acids (SFAs), four kinds of monounsaturated fatty acids (MUFAs), and five types of polyunsaturated fatty acids (PUFAs). The total content of free fatty acids (FFAs) in the groups inoculated with *D. hansenii* Y61 and Y67 increased by 54.53% and 77.31%, respectively. This increase can be attributed to the lipases secreted by yeasts [21]. Compared to the control group, the Y61 group showed an increase of 11.37% in SFAs, 54.60% in MUFAs, and 163.80% in PUFAs. On the other hand, the Y67 group exhibited a decrease of 11.69% in SFAs and an increase of 120.30% in MUFAs and 188.85% in PUFAs. The inoculation of *D. hansenii* Y61 and Y67 significantly increased the MUFAs and PUFAs content in the two batches of fermented sausages, thereby enhancing their nutritional value and aroma. Lipid-derived aroma compounds can be generated through auto-oxidation, and FFAs serve as the substrate for lipid oxidation. Therefore, the production of FFAs, to some extent, promotes the development of flavor in fermented sausage [26].

Table 4. The free fatty acids profile and content in different batches of sausages ripening for 18 days (mg/100 g lipid).

FFA	Group		
	Control	***D. hansenii* Y61**	***D. hansenii* Y67**
C10:0	34.30 ± 0.15 ^{a}	5.92 ± 0.24 ^{b}	0 ^{c}
C12:0	27.41 ± 0.45 ^{b}	65.07 ± 0.48 ^{a}	13.85 ± 2.70 ^{c}
C14:0	204.57 ± 1.80 ^{a}	269.98 ± 1.68 ^{b}	90.68 ± 10.03 ^{c}
C16:0	274.85 ± 0.15 ^{a}	230.228 ± 3.12 ^{b}	197.65 ± 2.43 ^{c}
C18:0	68.14 ± 0.45 ^{a}	23.668 ± 0.48 ^{c}	36.92 ± 2.70 ^{b}
C20:0	17.92 ± 4.80 ^{c}	103.64 ± 0.24 ^{b}	214.76 ± 5.40 ^{a}
∑SFA	627.19 ± 7.80 ^{a}	698.48 ± 8.40 ^{b}	553.86 ± 18.90 ^{c}
C14:1 n-5	18.68 ± 0.30 ^{a}	15.85 ± 1.20 ^{c}	19.28 ± 0.81 ^{b}
C16:1 n-3	45.63 ± 1.80 ^{a}	27.21 ± 28.08 ^{b}	30.14 ± 2.97 ^{c}
C18:1 n-9	497.80 ± 19.05 ^{c}	802.11 ± 36.24 ^{b}	1184.81 ± 40.50 ^{a}
C20:1 n-9	94.63 ± 11.55 ^{c}	170.12 ± 32.16 ^{b}	212.58 ± 4.32 ^{a}
∑MUFA	656.74 ± 32.7 ^{b}	1015.31 ± 97.68 ^{b}	1446.81 ± 48.60 ^{a}
C18:2 n-6	91.41 ± 0.75 ^{c}	143.86 ± 28.32 ^{b}	176.74 ± 35.37 ^{a}
C20:2 n-6	33.23 ± 0.30 ^{c}	75.95 ± 5.04 ^{b}	109.96 ± 3.24 ^{a}
C20:5 n-3 (EPA)	72.12 ± 0.15 ^{c}	123.75 ± 2.88 ^{b}	165.88 ± 13.50 ^{a}
C22:2 n-6	43.33 ± 0.15 ^{b}	85.42 ± 1.20 ^{a}	52.94 ± 0.54 ^{c}
C22:6 n-3 (DHA)	22.51 ± 0.45 ^{c}	170.12 ± 0.24 ^{b}	196.02 ± 2.70 ^{a}
∑PUFA	247.29 ± 1.80 ^{c}	652.34 ± 40.08 ^{a}	714.30 ± 55.89 ^{b}
Total	1531.22 ± 84.60 ^{c}	2366.13 ± 285.36 ^{b}	2714.97 ± 250.60 ^{a}

The values are the mean ± standard deviation. On the same row, the means with different letters differed significantly ($p < 0.05$), $n = 5$. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

It is widely accepted that various animal-derived FFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have broad-spectrum antimicrobial activities [34].

The bioconverted extracts of EPA and DHA have been shown to inhibit both Gram-positive and Gram-negative bacteria, including *S. enteritidis*, *Salmonella typhimurium*, *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus*. Additionally, DHA has been found to be more effective against Gram-negative bacteria compared to EPA. [34]. Therefore, the higher content of EPA and DHA found in the two yeast-inoculated groups could potentially inhibit the growth of spoilage bacteria, thereby extending the shelf life and enhancing the food safety of sausages.

3.5. Flavor

The table in Table 5 displays the relative contents of volatile substances in the three batches of sausages. In sausages treated with natural fermentation, a total of 26 different volatile substances were identified. On the other hand, sausages from the *D. hansenii* Y61 and Y67-inoculated groups had 34 and 43 different volatile substances, respectively. These volatile substances were categorized into eight groups, namely, aldehydes, ketones, alcohols, acids, ester compounds, alkanes, nitrogen compounds, and sulfur compounds. Notably, compared to other volatile compounds, the highest contents were observed in ester compounds and acids.

Table 5. Volatile compounds identified and quantified by gas chromatography–mass spectrometry of fermented sausages ripening for 18 days inoculated with *D. hansenii* strains (%).

Number	Volatile Compound	Group		
		Control	*D. hansenii* Y61	*D. hansenii* Y67
	Aldehydes			
1	Hexanal	11.4 ± 0.01 ^{a}	2.65 ± 0.12 ^{b}	1.17 ± 0.02 ^{c}
2	2-Heptenal	nd ^{b}	nd ^{b}	1.11 ± 0.15 ^{a}
3	2-Octenal	nd ^{b}	nd ^{b}	1.15 ± 0.02 ^{a}
	Total	11.4 ± 0.01 ^{a}	2.65 ± 0.12 ^{c}	3.43 ± 0.19 ^{b}
	Ketones			
4	2,2-dimethyl-Cyclobutanone	nd ^{b}	nd ^{b}	1.1 ± 0.01 ^{a}
5	3,4-Hexanedione	2.12 ± 0.03 ^{a}	0.29 ± 0.05 ^{b}	nd ^{c}
6	3,5-Octadien-2-one	1.22 ± 0.01 ^{b}	1.14 ± 0.11 ^{a}	0.47 ± 0.05 ^{c}
7	Pyrimidine-2,4-dione	nd ^{b}	nd ^{b}	0.38 ± 0.01 ^{a}
8	5,8-Quinolinedione	nd ^{b}	0.44 ± 0.01 ^{a}	nd ^{b}
	Total	3.78 ± 0.04 ^{ab}	2.16 ± 0.17 ^{a}	1.95 ± 0.07 ^{c}
	Alcohols			
9	5-methyl-2-Heptanol	8.11 ± 0.10 ^{a}	nd ^{b}	nd ^{b}
10	1,6-Octadien-3-ol	1.73 ± 0.12 ^{a}	0.6 ± 0.01 ^{c}	0.93 ± 0.01 ^{b}
11	Ethanol	4.07 ± 0.02 ^{a}	2.1 ± 0.01 ^{c}	2.46 ± 0.12 ^{b}
12	1-Nonanol	nd ^{b}	0.99 ± 0.03 ^{a}	nd ^{b}
	Total	13.91 ± 0.24 ^{a}	3.69 ± 0.05 ^{b}	3.39 ± 0.13 ^{c}
	Acids			
13	Formic acid	26.78 ± 0.15 ^{a}	nd ^{b}	nd ^{b}
14	2-methyl-Butanoic acid	0.78 ± 0.01 ^{a}	nd ^{b}	nd ^{b}
15	Acetic acid	1.1 ± 0.01 ^{c}	21.61 ± 0.41 ^{b}	24.67 ± 0.32 ^{a}
16	Benzoic acid	0.45 ± 0.01 ^{a}	nd ^{b}	nd ^{b}
17	Propanoic acid	0.67 ± 0.05 ^{c}	4.25 ± 0.01 ^{a}	3.27 ± 0.01 ^{b}
18	Propanedioic acid	nd ^{c}	14.09 ± 0.1 ^{a}	8.56 ± 0.10 ^{b}
19	2-ethyl-Heptanoic acid	nd ^{b}	0.37 ± 0.01 ^{a}	nd ^{b}
20	Lactic acid	0.79 ± 0.30 ^{c}	2.76 ± 0.12 ^{b}	3.44 ± 0.10 ^{a}
21	Pentanoic acid	nd ^{b}	nd ^{b}	0.31 ± 0.10 ^{a}
22	Mercaptoacetic acid	nd ^{c}	0.45 ± 0.01 ^{a}	0.36 ± 0.05 ^{b}
23	2-Cyclopentene-1-carboxylic acid	nd ^{b}	nd ^{b}	0.11 ± 0.03 ^{a}
24	1,2-Cinnolinedicarboxylic acid	nd ^{b}	nd ^{b}	0.7 ± 0.12 ^{a}
25	trans-Cinnamic acid	nd ^{b}	nd ^{b}	0.48 ± 0.01 ^{a}
26	Methylphosphonic acid	nd ^{b}	nd ^{b}	0.21 ± 0.07 ^{a}
27	5-Aminovaleric acid	nd ^{b}	0.76 ± 0.03 ^{a}	nd ^{b}
	Total	30.57 ± 0.23 ^{c}	44.29 ± 0.78 ^{a}	42.21 ± 0.91 ^{b}
	Ester compounds			
28	Hexanoic acid, ethyl ester	15.42 ± 0.12 ^{a}	18.19 ± 0.13 ^{b}	11.53 ± 0.07 ^{c}
29	Octanoic acid, ethyl ester	nd ^{c}	1.69 ± 0.04 ^{a}	0.82 ± 0.06 ^{b}
30	Acetic acid, butyl ester	nd ^{c}	0.58 ± 0.12 ^{b}	1.41 ± 0.07 ^{a}

Table 5. *Cont.*

Number	Volatile Compound	Group		
		Control	*D. hansenii* Y61	*D. hansenii* Y67
31	Decanoic acid, ethyl ester	nd ^{b}	nd ^{b}	3.84 ± 0.30 ^{a}
32	Isobutyl ester	nd ^{b}	0.4 ± 0.12 ^{a}	nd ^{b}
33	Butyrolactone	nd ^{c}	3.22 ± 0.05 ^{b}	1.96 ± 0.10 ^{a}
34	Ethyl 9-hexadecenoate	nd ^{b}	0.88 ± 0.11 ^{a}	nd ^{b}
35	Isoamyl lactate	nd ^{b}	nd ^{b}	0.18 ± 0.01 ^{a}
36	Hexadecanoic acid, ethyl ester	nd ^{b}	0.24 ± 0.01 ^{a}	nd ^{b}
37	Methoxyacetic acid, butyl ester	nd ^{c}	7.22 ± 0.03 ^{b}	13.82 ± 0.12 ^{a}
38	Hexanoic acid, methyl ester	nd ^{b}	nd ^{b}	2.63 ± 0.21 ^{a}
39	Octanoic acid, ethyl ester	nd ^{c}	0.64 ± 0.03 ^{b}	0.82 ± 0.01 ^{a}
40	Heptanoic acid, ethyl ester	nd ^{b}	nd ^{b}	1.67 ± 0.12 ^{a}
41	Pentanoic acid, ethyl ester	0.86 ± 0.01 ^{b}	0.78 ± 0.21 ^{c}	1.77 ± 0.05 ^{a}
42	Trimethylsilyl ester	1.4 ± 0.03 ^{a}	1.4 ± 0.01 ^{a}	0.74 ± 0.02 ^{b}
43	Formic acid, hept-2-yl ester	nd ^{b}	0.4 ± 0.06 ^{a}	nd^{b}
44	3,4-Hexanedione, 2,2,5-trimethyl	4.12 ± 0.02 ^{b}	4.35 ± 0.10 ^{a}	nd ^{c}
45	Pentafluoropropionate	nd ^{b}	0.6 ± 0.01 ^{a}	nd ^{b}
46	4-Methylcyclohexanol acetate	nd ^{b}	0.36 ± 0.05 ^{a}	nd ^{b}
47	Fumaric acid, nonylmtetrahydrofurfuryl ester	0.67 ± 0.03 ^{b}	2.12 ± 0.03 ^{a}	nd ^{c}
	Total	22.47 ± 0.21 ^{c}	42.05 ± 1.08 ^{b}	41.19 ± 1.14 ^{a}
	Alkanes			
48	1,4-dichloro-Cyclohexane	nd ^{b}	nd ^{b}	0.18 ± 0.03 ^{a}
49	Cyclopentasiloxane	3.4 ± 0.02 ^{a}	nd ^{c}	1.68 ± 0.01 ^{b}
50	Dodecamethyl-pentasiloxane	0.98 ± 0.13 ^{a}	0.79 ± 0.05 ^{b}	0.58 ± 0.10 ^{c}
51	Heptasiloxane	3.76 ± 0.12 ^{a}	nd ^{b}	nd ^{b}
52	Difluorodimethyl-silane	0.1 ± 0.03 ^{b}	nd ^{c}	0.22 ± 0.01 ^{a}
53	3-Methyl-oxiran	nd ^{b}	nd ^{b}	1.36 ± 0.17 ^{a}
	Total	8.24 ± 0.30 ^{a}	0.79 ± 0.05 ^{c}	4.02 ± 0.37 ^{b}
	Sulphur compounds			
54	3-Amino-2-phenazinol ditms	nd ^{b}	0.48 ± 0.12 ^{a}	0.23 ± 0.01 ^{b}
55	2-Furanylmethyl	nd ^{b}	nd ^{b}	1.05 ± 0.05 ^{a}
56	Tetrahydrofuran	nd ^{b}	nd ^{b}	0.41 ± 0.10 ^{a}
57	2-Methyl-tetrahydroquinoxaline	0.35 ± 0.20 ^{b}	0.14 ± 0.17 ^{c}	0.68 ± 0.03 ^{a}
58	2-pheny-1-Pyrroline	nd ^{b}	nd ^{b}	0.42 ± 0.06 ^{a}
59	4-phenyl-Quinazolin	1.96 ± 0.05 ^{a}	nd ^{b}	nd^{b}
60	Dimethyl ether	7.21 ± 0.01 ^{a}	2.75 ± 0.03 ^{b}	0.75 ± 0.15 ^{c}
61	N-Morpholinomethyl-isopropyl-sulfide	0.56 ± 0.05 ^{a}	nd ^{c}	0.27 ± 0.10 ^{b}
	Total	10.08 ± 0.31 ^{a}	3.37 ± 0.32 ^{c}	6.60 ± 0.50 ^{b}
	Nitrogen compounds			
62	N-dimethylpropanamide	nd ^{b}	nd ^{b}	0.38 ± 0.01 ^{a}
63	Formamide	5.43 ± 0.20 ^{a}	nd ^{b}	nd ^{b}
64	L-Alanine-4-nitroanilide	nd ^{b}	nd ^{b}	0.42 ± 0.24 ^{a}
	Total	5.43 ± 0.20 ^{a}	nd^{c}	0.80 ± 0.25 ^{b}

The values are the mean ± standard deviation. On the same row, the means with different letters differed significantly ($p < 0.05$), $n = 5$.

Among the volatile compounds detected in the two yeast-inoculated groups, the most abundant substances were ester compounds, which accounted for 42.19% and 41.19% in Y61 and Y67, respectively. These findings are consistent with previous studies that have reported the potential of yeast strains to produce aroma through the generation of ester compounds [13,15]. Studies have shown that ester compounds were produced through the esterification of alcohols and acids, including linear acids, branched acids, and unsaturated acids [15,35]. In non-inoculated fermented sausages, only 5 kinds of ester compounds were detected, while 16 and 12 ester compounds were detected in the *D. hansenii* Y61 and Y67 groups, respectively. Compared with the control group, the total amount of ester compounds increased by 87.14% and 83.31%, respectively. This increase strongly contributed to the characteristic "fruity", "dry-cured," and "fresh" aroma of fermented sausages [15]. Among the ester compounds detected in the *D. hansenii*-inoculated groups, hexanoic acid and ethyl ester comprised the highest concentrations, accounting for 18.19% and 11.53%, respectively. Previous studies reported that ester production was strongly dependent on the yeast strain [35]. Therefore, it is important to note that Y61 and Y67 *D. hansenii* could be valuable for enhancing the aroma of fermented sausages.

The aldehydes in the fermented sausages of the Y61 and Y67 *D. hansenii*-inoculated groups were mainly hexanal. These levels were reduced by 76.75% and 89.74%, respectively, compared to the naturally fermented sausages (control group). Hexanal is known to be produced through the cleavage of hydroperoxide, which is formed by the oxidation of linoleic acid. It serves as an indicator of the degree of oxidation [26]. In the present study, the decrease in hexanal content in the Y61 and Y67-inoculated groups corresponded to the lower levels of lipid oxidation. These findings confirmed the antioxidant effects of *D. hansenii*, as reported in other reports [15]. Additionally, with the decreases in lipid oxidation of yeast-fermented sausages, the shelf life and edibility of fermented sausages were increased.

Compared with naturally fermented sausages, the contents of alcohols and alkanes in the *D. hansenii* Y61 and Y67 inoculated groups decreased (Table 5). Studies have shown that alcohols and alkanes in meat might be the product of auto-oxidation of lipids, but because of their higher threshold [36], they could contribute less to the aroma of fermented sausages.

As shown in Table 5, 7 and 10 kinds of acid substances were detected in the fermented sausages of the Y61 and Y67 *D. hansenii*-inoculated groups, which increased by 44.88% and 38.07%, respectively. Among them, short-chain organic acids such as acetic acid (24.67% vs. 1.1%), propanedioic acid (8.56% vs. 0), propanoic acid (3.27% vs. 0.67%), and lactic acid (3.44% vs. 0.79%) of the Y61 inoculation group were significantly increased, when compared with the control group, while the content of formic acid was significantly reduced. Compared to the control group, short-chain organic acids such as acetic acid (24.67% vs. 1.1%), propanedioic acid (8.56% vs. 0), propanoic acid (3.27% vs. 0.67%), and lactic acid (3.44% vs. 0.79%) of the Y67 *D. hansenii*-inoculated group were significantly increased, while the content of formic acid was significantly reduced. Studies have shown that organic acid substances are mainly derived from lipid degradation, amino acid deamination, or metabolites from microbial growth and reproduction [36]. It is generally believed that some low-molecular-weight organic acids such as acetic acid, propionic acid, and butyric acid are derived from microorganisms [33,36]. Our previous studies have reported that the two strains of *D. hansenii* had strong acid-producing ability, and the increase in acid content of yeast-inoculated groups was likely derived from the action of yeasts. In addition, the short-chain organic acids could contribute to the good flavor of fermented sausage and inhibit the reproduction of harmful microorganisms [33,36].

3.6. Sensory Evaluation

The effects of inoculation of Y61 and Y67 *D. hansenii* on the sensory quality of fermented sausages are shown in Figure 1. The groups with the added starter showed higher scores than the control group in terms of appearance, flavor, texture, taste, and overall acceptability. Figure 1 also illustrates that the main contributions of the inoculation groups to the sausages were in terms of taste and flavor. The taste scores of the Y61 and Y67 inoculation groups were an average of 8.0 and 7.4, respectively, which were higher than that of the control of 31.3% and 25.5%, respectively. The flavor scores of the Y61 and Y67 inoculation groups were 8.20 and 7.20, respectively, which were 37.9% and 29.2% higher than those of the control group, respectively. It was suggested that *D. hansenii* Y61 and Y67 could contribute to a special flavor and add taste to the sausages. Furthermore, the inoculated yeasts improved the appearance, integrity, and texture of the fermented sausages. Based on Figure 1's results, inoculation of Y61 and Y67 *D. hansenii* improved the overall acceptance of the sausages, and the overall acceptability of the sausages from the group of Y61 was higher, with a score of 8.0. Comparing the effects of the two strains, the quality of sausages inoculated with *D. hansenii* Y61 was better than *D. hansenii* Y67.

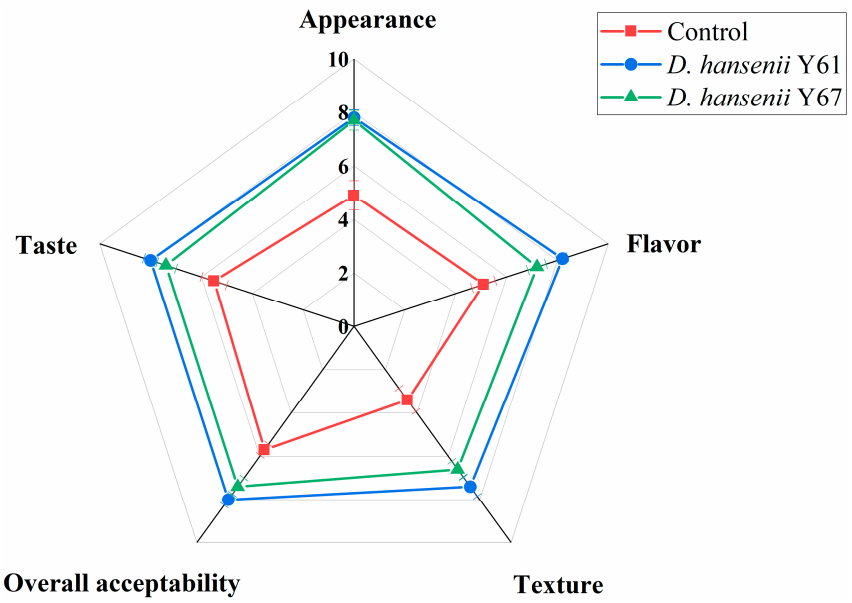

Figure 1. Sensory evaluation of fermented sausages ripening for 15 days.

4. Conclusions

Inoculation of the Y61 and Y67 strains of *D. hansenii* affected the ripening process by decreasing the pH and a_w. Sausages inoculated with Y61 and Y67 *D. hansenii* resulted in decreased lipid oxidation levels (TBARS), *Escherichia coli* counts, and improved color stability and texture properties. The Y61- and Y67-inoculated *D. hansenii* sausages resulted in higher levels of free amino acids (FAAs) and free fatty acids (FFAs) through lipid and protein decomposition, which improved the sensory evaluations and safety of sausages. Moreover, both yeast strains were responsible for the generation of esters (accounting for 42.19% and 41.19%) and acid compounds (accounting for 44.29% and 41.21%) in the fermented sausages. The Y61 and Y67 strains of *D. hansenii* were good aroma enhancers in fermented sausages through increases in ester and acidic compounds. Furthermore, better sensory attributes were found in sausages inoculated with Y61 and Y67 *D. hansenii*. Consequently, Y61 and Y67 *D. hansenii* were good starter cultures for fermented sausage production. The results of this study can serve as a reference to enrich the bank of starter cultures for fermented meat products and provide more information about the use of indigenous yeasts in the fermentation of traditional Chinese meat products like sausages.

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