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Effect of *Lactobacillus plantarum* with antioxidant properties on the flavour and oxidation of Chinese sausages

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Abstract: Effects of *Lactobacillus plantarum* (*L. plantarum*) strain P3 and mutant strain P3-M2 with antioxidant properties on fermented sausage flavour via lipid and protein oxidation inhibition were investigated. The commercial strain was used to as positive control (control group). Results showed that P3 and P3-M2 had the ability of reducing lipid and protein oxidation during fermentation. The increase of lipoxigenase activity and thiobarbituric acid reactive substances (TBARS) values were retarded. Metmyoglobin (MetMb) content relatively decreased significantly ($p < 0.05$), while sulfhydryl group contents were significantly higher than those in the control ($p < 0.05$). Furthermore, changes in protein bands were confirmed with the less protein oxidation with P3-M2 than P3 and the control. Additionally, strain P3 and P3-M2 significantly enhanced the type and relative content of esters after fermented ($p < 0.05$), indicating that strain P3 and P3-M2 contributed to the production of flavor substances. These results revealed that *L. plantarum* strains with antioxidant properties were a promising approach in inhibiting lipid and protein oxidation of Chinese sausage, maintaining the stable natural structure of protein, simultaneously improve the quality of sausage and promote the sausage to form a better flavor.

Keywords: *Lactobacillus plantarum*; Chinese sausage; antioxidation; flavour; quality

1. Introduction

Chinese fermented sausage is a popular traditional meat product widely preferred by consumers because of its characteristic flavour [1]. Lactic acid bacteria (LAB) and their fermentation products are generally recognised as safe, guaranteeing that have been widely utilised in food [2]. In recent years, an increasing number of researchers have focused on LAB fermentation to improve flavour and ensure food safety.

Many effect of LAB on flavour substances have been reported. The higher amount of volatile compounds and organic acids due to the adjunct *Lactobacillus paracasei* 4341 led to a clear differentiation of the experimental Caciotta compared to the control in terms of aromatic profile, colour, texture, and sensory perception [3]. A co-culture of *L. harbinensis* M1 and *L. casei* M8 produced a fermented soymilk product with both markedly improved flavour and good probiotic potential [4]. *Lactobacillus*, the dominant bacteria in fermented rice noodles, grow rapidly to inhibit the growth of undesirable bacteria and produce various low-threshold volatile compounds, including aldehydes, esters, alcohols, and ketones. A significant correlation between bacterial communities and major volatile compounds in dry sausages was found, and the core bacteria, including *Lactococcus lactis*, *L. plantarum*, *L. alimentarius*, and *L. sakei* and *Weissella hellenica*, contributed to the development of the major volatile compounds [5]. Thus, LAB

fermentation contributes to the production of complex flavour components, such as amino acids, vitamins, and organic acids, which improve the flavour, taste, and other properties of fermentation broth [6].

Oxidation is one of the main causes of deterioration of Chinese fermentation sausages and may cause taste, texture, discolouration and other undesirable effects, such as loss of essential fatty acids and amino acids [7,8]. Therefore, the lipid and protein oxidation during the manufacturing and storage of meat products, is still a major research topic in food technology [9,10]. Both protein oxidation and lipid oxidation are related to the formation of sausage quality, which is the most remarkable characteristic and important index for quality evaluation [11]. Lipid oxidation greatly affects the quality of meat products and is the main factor limiting the shelf life and flavour of products. During lipid oxidation, secondary oxidation products such as aldehydes, ketones, and acids are produced. These products can contribute to the formation of the unique flavour of sausage but result in an unpleasant smell, even producing malondialdehyde (MDA) and other harmful substances when excessive oxidation occurs [12]. Protein oxidation can occur during sausage fermentation and maturation, potentially changing the structural and functional properties of the proteins [13]. This also has important effect on the quality, nutritional value, and sensory characteristics of fermented sausages [14].

However, effects of the application of *L. plantarum* with antioxidant properties as a starter culture to mediate the flavour and oxidation of sausage during fermentation remain unclear. Therefore, this study aimed to investigate the effects of *L. plantarum* as a fermentation strain on the flavour of sausages by regulating protein and lipid oxidation during fermentation and ripening.

2. Materials and Methods

2.1 Materials and chemicals

Pork fat and lean pork were purchased from Hongfu Supermarket (Hefei, Anhui Province, China). Before each trial of sausage preparation, pork meat was trimmed to remove visible connective tissues and fat. The sausages were prepared using 70% pork meat and 30% pork fat in a total mass of 50 kg. After the pork meat and pork fat were minced, the ingredients including salt (3%, w/w), sugar (7%, w/w), sodium glutamate (0.2%, w/w), five flavoured powder (0.1%, w/w), ginger powder (0.15%, w/w), and Daqu liquor (2%, v/w) were added. The five flavoured powder and ginger powder were purchased from Hongfu Supermarket (Hefei, Anhui Province, China). The mixture was divided into three groups. The first group was set as the control group by adding 200 mg/kg of the commercial strain *Lactobacillus* Speed 1.0. The second group was added with 200 mg/kg of *L. plantarum* strain P3 (P3 group). The third group was added with 200 mg/kg of mutant strain P3-M2 (P3-M2 group).

The mixtures were stuffed in natural casings with a 4-cm diameter and 10-cm length. All treatments were performed in three different batches for experimental replication, and sausages were placed in a fermentation room and ripened for three periods. In the first stage of fermentation, the relative humidity (RH) and temperature were set at 95% and $30 \pm 0.5^\circ\text{C}$, respectively, for 1 day. Then, the temperature was adjusted to $16 \pm 0.5^\circ\text{C}$, and RH was successively decreased to 90%, 87%, and 85%, and the duration of each RH was 2 days. The fermentation temperature of the third stage was $12 \pm 0.5^\circ\text{C}$, and the RH was set at 85%, 80%, and 75%, and each RH condition lasted 7 days. The total ripening period was 28 days. Samples were taken from each group at 4, 10, 16, 22, and 28 d of fermentation. DPPH was purchased from Beijing Zhongsheng Ruitai Technology Co., Ltd., O-phenanthroline was purchased from Tianjin Fu Technology Development Co., Ltd., K_2HPO_4 , KH_2PO_4 , NaH_2PO_4 , NaCl, and H_2O_2 were obtained from Xilong Chemical Co., Ltd., pyrogallol, potassium ferricyanide, trichloroacetic acid were obtained from Shanghai McLean Biological Reagent Co., Ltd., and the T-AOC kit, carbonyl

determination kit, and sulfhydryl determination kit were obtained from Nanjing Jiancheng Bioengineering Research Institute (Nanjing, Jiangsu Province, China).

2.2 Bacterial cultures and culture media

One *L. plantarum* strain P3 was previously isolated from Chinese sausages and identified by 16S rDNA sequencing was used in this study. P3-M2 was a mutant strain of P3 and was identified by 16S rDNA sequencing. De Man, Rogosa, Sharpe (MRS) agar and MRS broth used to culture strain were obtained from Hangzhou Best Bio Technology Co., Ltd.

2.3 Determination of free radical scavenging ability

2.3.1 Hydroxyl radical-scavenging assay

The hydroxyl radical (OH)-scavenging assay was performed according to the method described by Noureen et al.[15] with some modifications. The reaction mixture contained 1.0 mL of ethanolic salicylic acid solution (5 mM), 1.0 mL of FeSO₄ (2.5 mM) and 1.0 mL of H₂O₂ (20 mM). The solution was mixed with 1.0 mL of the sample, and then deionised water was added to a volume of 10 mL. The mixture was incubated at 37°C for 90 min, and the absorbance of the resulting solution was measured at 536 nm after centrifugation at 6000 × g for 10 min. The control group contained an equal volume of deionised water instead of the sample. The OH-scavenging activity was expressed as follows:

$$\text{Scavenging activity (\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100.$$

2.3.2 DPPH radical-scavenging assay

The DPPH free radical-scavenging capacity of *L. plantarum* was evaluated according to the method described by Pil-Nam et al. [16] with some modifications. Briefly, 2.0 mL of the sample was added to the same volume of an ethanolic DPPH radical solution (0.4 mM). The reaction solution was mixed vigorously and incubated at room temperature in the dark for 30 min. The control group contained an equal volume of deionised water instead of the sample. The blank group contained an equal volume of ethanol instead of DPPH radical solution. The absorbance of the solution was measured at 517 nm after centrifugation at 8000 × g for 10 min. The scavenging ability was defined as follows:

$$\text{Scavenging activity (\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100.$$

2.3.3 Ferrous ion-chelating ability

The Fe²⁺-chelating ability was estimated using the method described by Lin and Yen [17]. First, 0.5 mL of the sample was mixed with 0.1 mL of ascorbic acid (10 g/L), 0.1 mL of FeSO₄ (4 g/L), and 1 mL of NaOH (0.2 M). The mixture was incubated at 37°C in a water bath for 20 min, and 0.2 mL of TCA (10%) was added. The supernatant was obtained by centrifugation at 4500 × g for 10 min, and 1.5 mL of 1, 10-phenanthroline (1 g/L) was added. After allowing the reaction to proceed for 10 min, the absorbance was measured at 510 nm.

2.3.4 Superoxide anion -scavenging assay

The superoxide anion radical scavenging assay was performed using an improved pyrogallol autoxidation method [18]. First, 0.1 mL of the sample was added to 4.5 mL of Tris-HCl solution (0.05 M, pH 7.0). The reaction mixture was incubated at 25°C for 20 min. Subsequently, 0.4 mL of pyrogallol (2.5 mM) was added, and the mixture was incubated at room temperature for 4 min. Then, two drops of HCl (8 M) were added to stop the reaction, and the absorbance was measured at 306 nm. The control group

contained an equal volume of deionised water instead of the sample. The O₂ -scavenging activity is expressed as:

$$\text{Scavenging activity (\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / A_{\text{control}}] \times 100.$$

2.4 Preparation of the fermented sausage

Sausages were prepared according to the method described by Ge et al.[14] with some modifications. A total of 70 % pork meat and 30 % pork fat was minced, and the ingredients including salt (3 %, w/w), sugar (7 %, w/w), sodium glutamate (0.2 %, w/w), five flavoured powder (0.1 %, w/w), ginger powder (0.15 %, w/w), and Daqu liquor (2 %, v/w) were added. The mixture was divided into three groups. One group was set as the control with a commercial fermentation strain (strain *Lactobacillus* Speed 1.0). The other groups were supplemented with *Lactobacillus plantarum* (*L. plantarum*) P3 (P3 group) and *L. plantarum* P3-M2 (P3-M2 group).

2.5 Lipid oxidation

2.5.1 Determination of TBARS value of sausage

Among the various parameters used to reflect lipid oxidation, the most common is determining the TBAR value [19]. Lipid oxidation was assessed by the thiobarbituric acid reactive substances (TBARS) procedure [20] with some modifications. Chopped sausage (5 g) was blended with 50 mL of trichloroacetic acid mixed solution, sealed, and oscillated at 50°C for 30 min. The mixture was filtered using a double Whatman filter paper. Then, 5 mL of filtrate and 5 mL of 20 mM TBA were transferred into a tube, covered, and heated at 90°C for 30 min. The concentration of malondialdehyde (MDA, mg/kg sausage) was measured by determining the absorbance of the solution using a UV spectrophotometer at 532 nm of wavelength.

2.5.2 Determination of peroxide value of sausage

The lipid extraction method described by Ying et al. [21] was used. POV measurement was performed using the Chinese National Standard (GB/T 5538-2005). The lipid extract (5.0 g) was completely dissolved in 50 mL of component solvent (acetic acid: isooctane = 3:2, v/v), and saturated potassium iodide solution (0.5 mL) was added and placed in the dark for 3 min. Then, 30 mL of distilled water was added and titrated with 0.01 mol/L sodium thiosulfate solution to the mixture until the blue colour disappeared. A 0.5 mL of starch solution (1 %, m/v) was used as an indicator.

2.6 Protein oxidation

2.6.1 Protein preparation

Sarcoplasmic and myofibrillar protein extracts were prepared from sausages according to the method described by Di Yu et al. [22] with some modifications. A 10 g of chopped sausage was homogenized with a 100 mL 0.03 M, pH 6.5 Na₂HPO₄-NaH₂PO₄ buffer by high-speed homogeniser (IKA, Staufen, Germany) for 3 min, centrifuged at 8000 rpm/min for 20 min at 4°C. The supernatant was dialysed in distilled water to obtain sarcoplasmic protein. The precipitate was homogenised with a 0.03 M, pH 6.5 Na₂HPO₄-NaH₂PO₄ buffer at a mass-to-volume ratio of 1: 10 (W/V) for 3 min, centrifuged at 8000 rpm for 20 min at 4°C, and was repeated twice. The precipitate was homogenised with a 0.1 M, pH 6.5 Na₂HPO₄-NaH₂PO₄ buffer containing 0.7 M KI and 0.02 % NaN₃ at a mass-to-volume ratio of 1: 4 (W/V), centrifuged at 10000 rpm for 20 min at 4°C. The supernatant was dialysed in distilled water to obtain myofibrillar protein. The concentration of the obtained protein was determined using a TP kit.

2.6.2 Carbonyl content

The carbonyl group was determined according to a previously reported method with slight modifications [23]. MPs were diluted to a 5 mg/mL with 15 mM PIPES buffer (15 mM PIPES, 600 mM NaCl, pH 6.0). Two groups of 0.8 mL MP solutions were obtained. One was treated with 1.6 mL of 3 mM HCl containing 0.3% (w/v) 2, 4-dini-trophenylhydrazine, and the other was treated with 1.6 mL of 3000 mM HCl. After the solutions were reacted for 30 min at room temperature, 0.8 mL of trichloroacetic acid (40%, w/v) was added to each solution and centrifuged (2500 × g, 5 min, 4 °C). The supernatant was removed. Next, 2 mL of the mixture (ethanol: ethyl acetate = 1: 1) was mixed with the precipitate, centrifuged under the same conditions, and washed three times. The precipitate was dissolved by adding 3 mL of 20 mM polarisation beam splitter (PBS) (pH 6.5) containing 600 mM guanidine hydrochloride. The absorbance was measured at 370 nm, and the protein carbonyl content was determined using the molar extinction coefficient (22,000 M⁻¹ cm⁻¹) as follows:

$$\text{Carbonyl content (nmol/mg)} = \frac{3 \times A \times 10^5}{C \times v \times 22000}$$

2.6.3 Sulfhydryl content

The total sulfhydryl content was determined according to previously described methods [24]. MPS was diluted to 10 mg/mL with 15 mM PIPES buffer (15 mM PIPES, 600 mM NaCl, pH 6.0). Then, 3 mL of the diluted solution was mixed with 0.4 mL of 0.1% 2-nitrobenzoic acid, left at 40°C for 25 min in the dark, and then cooled to room temperature. Absorption was measured at a wavelength of 412 nm. The formula used was as follows:

$$\text{Sulfhydryl content (nmol/mg)} = \frac{A \times 10^5}{136 \times C}$$

where A is the absorbance and C was the protein concentration.

2.6.4 MetMb analysis

MetMb content was determined according to the method described by Samaoui et al. [25]. Chopped sausage (5 g) was homogenised with a 25 mL iced PB buffer (0.04 M, pH 6.8) for 10 s, placed at 4 °C for 1 h, centrifuged at 4500 rpm for 30 min, and the supernatant was filtered with a filter paper. The absorbance of the filtrate was measured using a UV spectrophotometer at wavelengths of 525, 545, 565, and 572 nm. The following formula was used:

$$\text{MetMb \%} = [-2.51(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098] \times 100.$$

2.6.5 SDS-PAGE

The degradation of the extracted sarcoplasmic protein was analysed according to the method described by Chen et al. [26]. The protein was diluted to a concentration of approximately 1 mg/mL, and an equal volume of sample loading buffer was mixed with the protein solution and then heated at 100°C for 5 min. A 12% polyacrylamide separating gel was used to detect protein bands. Twenty microliters of the prepared samples were loaded into each well and run at 80 V for 30 min and then at 100 V for 60 min. The gels were stained with a buffer containing 45% methanol, 10% glacial acetic acid, and 0.1% (m/v) coomassie brilliant blue for 30 min and destained with distilled water overnight until emerging the clean background. Protein bands were scanned using a scanner.

2.7 Volatile substance analysis

The sample was processed by the solid-phase microextraction method described by Song Y et al. [27]. Chopped sausage (5 g) was placed in a 20 mL headspace bottle and pressed to 1/3 of the bottle, and cyclohexanone was added as the internal standard. The aging extraction head was inserted into the headspace of the sample bottle and adsorbed at 80°C for 60 min, after which the adsorbed extraction head was removed, inserted into the gas chromatograph inlet, and analysed at 250°C for 10 min, after which the data were collected and collected again after 50 min. Chromatographic conditions were as follows: a TR-5 MS capillary column (30 m × 0.25 mm × 0.25 µm), helium was used as the carrier gas, and the flow rate was set at 1 mL/min. Heating program: the furnace temperature was maintained at 40 °C for 3 min, and the temperature was raised to 90 °C at a heating rate of 5 °C/min without maintaining; then, the temperature was increased to 230 °C at a rate of 10 °C/min and maintained for 6 min. Mass spectrometry conditions were as follows: ion source temperature, 200 °C; ionisation mode, EI⁺; electron energy, 70 eV; emission current, 120 µA; scanning mass range was 30-50 m/z. The relative percentage of each flavour compound was calculated using the peak area normalisation method.

2.8 Statistical analysis

All experimental data were analysed using the software SPSS 24.0. Differences between groups were analysed by ANOVA, and multiple comparisons were performed using the Duncan method. Statistical significance was set at $p < 0.05$. Test data are expressed as the mean ± SD. Three batches of sausages (replicates) were prepared, and all measurements were triplicated for each batch of sausages.

3. Results and Discussion

3.1 Antioxidant ability of P3 and P3-M2

Hydroxyl radicals are highly oxidising and have a strong influence on all cellular components, including lipids, nucleic acids, and proteins. The free radical scavenging abilities of *L. plantarum* P3 and P3-M2 fermentation supernatants are presented in Figure 1. Mutant strain P3-M2 was obtained by mutation breeding of P3, with significantly improved comprehensive antioxidant capacity ($p < 0.05$). The hydroxyl radicals, DPPH radical scavenging ability, Fe²⁺ chelating ability, and superoxide anion scavenging ability (Figure 1 a, b, c, d) of the P3-M2 fermentation supernatant were all significantly improved ($p < 0.05$). The DPPH free radical scavenging ability was as high as 92.74%, the scavenging rate of *L. plantarum* P3-M2 on hydroxyl radicals was approximately 65%, and the scavenging rate of superoxide anion was approximately 40.74%. In particular, the Fe²⁺ chelating ability of P3-M2 was higher than that of P3 by approximately 51.01%. The T-AOC value (Figure 1e) of P3-M2 was 30.85 ± 0.67 U/mL, which was 33.78 % higher than that of P3 ($p < 0.05$). It was shown that the mutant strain P3-M2 significantly improved the ability to scavenge free radicals and antioxidant capacity.

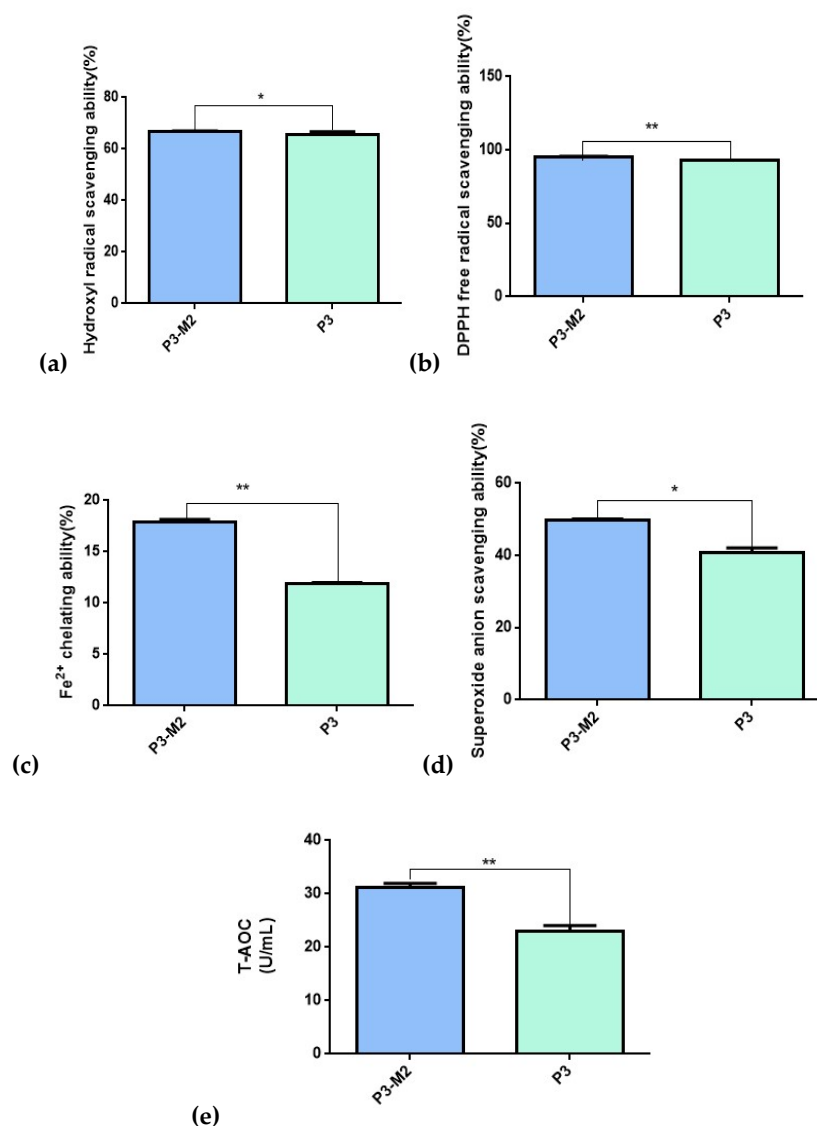


Figure 1. Free radical scavenging ability of fermentation supernatant from *Lactobacillus plantarum* P3-M2 and P3. (a)The hydroxyl radicals ability of the P3-M2 fermentation supernatant, (b)The DPPH radical scavenging ability of the P3-M2 fermentation supernatant, (c)The Fe²⁺ chelating ability of the P3-M2 fermentation supernatant, (d)The superoxide anion scavenging ability of the P3-M2 fermentation supernatant, (e)The Total AOC(T-AOC) of the P3-M2 fermentation supernatant. * above the bars indicate statistically significant differences ($p < 0.05$).

3.2. Lipid oxidation

3.2.1. TBARS value

In gradual oxidation of fat, aldehydes, ketones, acids, and other secondary oxidation products were produced, forming a unique sausage flavour. However, excessive lipid oxidation leads to meat odour [12] and generates malondialdehyde (MDA) and other harmful substances, which greatly affect the quality of meat products. This was the main factor limiting the shelf life of the products. TBARS is the most direct index for evaluating the oxidation of meat products worldwide.

As shown in Figure 2, the TBARS values of sausages gradually increased, indicating that lipid oxidation occurred. There was no significant difference between the three groups before day 16 ($p \geq 0.05$).

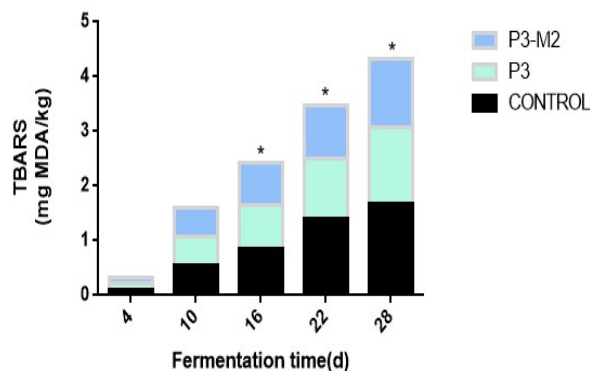


Figure 2. Changes of TBARS values of three groups sausages during fermentation. Data are expressed as the mean of triplicate samples \pm standard error. Vertical bars represent the standard errors of the means. * above the bars indicate statistically significant differences ($p < 0.05$).

On day 4, the TBARS of the three groups was 0.11 mg MDA/kg. However, On the day 10 the TBARS values of P3 group and P3-M2 group were 0.48 mg MDA/kg and 0.50 mg MDA/kg respectively, which were significantly lower than those of control group ($p < 0.05$). After day 10, the TBARS values of the three groups were as follows: P3-M2 group < P3 group < Control group. The lower TBARS values of P3-MS were significantly different ($p < 0.05$) after day 16. At the end of storage, the TBARS values in the P3-M2 group were 1.25 mg MDA/kg lower than those in the control and P3 groups ($p < 0.05$). This showed that LAB inhibited the access to sausages with the production of MDA. This was consistent with the results of Libera et al. [28], who reported that the addition of probiotic strain reduced lipid oxidation.

Antioxidants inhibit fat oxidation mainly by scavenging free radicals or chelating metal ions to prevent free radical chain reactions. Strains P3 and P3-M2 have a strong ability to scavenge free radicals, such as DPPH free radicals, hydroxyl free radicals, and superoxide anions, which can achieve the effect of delayed lipid oxidation. The good scavenging activity of *lactic acid bacteria* against free radicals has also been confirmed in other studies [29]. Di Yu [22] showed that *L. plantarum* and *Staphylococcus simulans* inoculated into fermented sausage contributed to the generation of antioxidant peptides and thus exhibited good antioxidant activity.

3.2.2 Changes of lip oxygenase (LOX) activity

LOX is an enzyme that catalyses the oxidation of polyunsaturated fatty acids to produce hydroperoxides. It acts on the 1, 4-cis, cis-pentadiene group positions of unsaturated fatty acids to lose protons and form lipid free radicals.

Changes in LOX activity are shown in Figure 3. LOX activity gradually decreased with the extension of fermentation time ($p < 0.05$). The results were consistent with Bian et al. [30] that the early stage of sausage lip oxidation was mainly affected by enzyme oxidation. LOX plays a role in the loss of unsaturated fatty acid protons and the formation of lipid-free radicals. Thus, these free radicals could induce automatic oxidation of fat, whereas in the later stage, the automatic oxidation was mainly caused by free radicals. The LOX activity of sausages in the P3-M2 group was significantly lower than that in the P3 group on day 4 ($p < 0.05$). The LOX activity of the P3-M2 group sausage was significantly higher than that of the control group and P3 group ($p < 0.05$), indicating that strains P3-M2 had the ability to inhibit LOX activity.

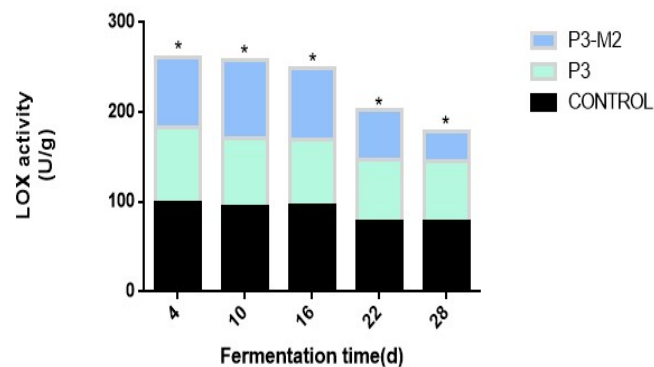


Figure 3. Changes of LOX activity in three groups of sausages of three groups during fermentation. Note: Different capital letters indicate significant differences in LOX activity between the three groups of samples ($p < 0.05$); * indicate significant differences in LOX activity during the same group of fermentation ($p < 0.05$).

Because LOX is an oxidoreductase, it essentially contains non-heme ferritin. The LOX of iron existed in the Fe^{2+} and Fe^{3+} states. The Fe^{2+} state was activated, whereas Fe^{3+} was the catalytic activity. From the radical scavenging results, it was also found that strain P3-M2 had a strong ability to chelate Fe^{2+} . Therefore, it could be inferred that the strains of P3-M2 could cooperate with Fe^{2+} , maintaining its inactive state to inhibit the activity of LOX.

3.3 Protein oxidation

3.3.1 Carbonyl content

Carbonyl is the oxidation product of amino acid side chains (such as proline, histidine, lysine, and arginine residues). The carbonyl content is proportional to the degree of protein oxidation [31]. The formation of carbonyl content can affect the nutrition, flavour, and texture of meat products. For example, carbonyl compounds of proteins can form specific Strecker aldehydes, which contribute to the flavour of meat products, but the oxidation of certain amino acids, including essential amino acids, can lead to a decrease in the nutritional value of meat [32].

The changes in the carbonyl content are shown in Figure 4. After 10 d, the carbonyl content of the P3-M2 group protein was significantly lower than that of the control group ($p < 0.05$) in both the sarcoplasmic protein (Figure 4a) and myofibrillar protein (Figure 4b), indicating that *L. plantarum* P3-M2 could reduce the formation of carbonyl groups in the protein. Ge et al. [14] also observed that *L. plantarum* NJAU-01 could be used as a starter in sausages to reduce carbonyl production during fermentation.

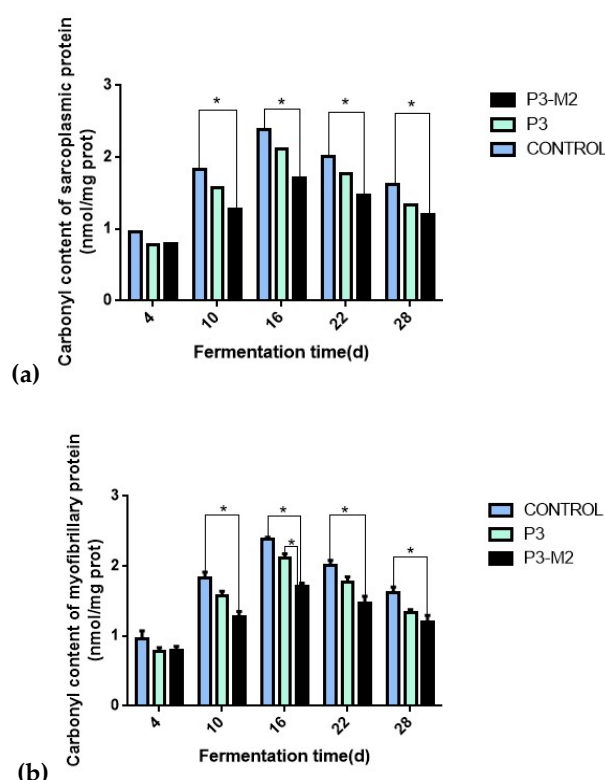


Figure 4. Changes of carbonyl content in sarcoplasmic protein(a) and myofibrillar protein(b) of three groups during fermentation. Data are expressed as the mean of triplicate samples \pm standard error. Vertical bars represent the standard errors of the means. * above the bars indicate statistically significant differences ($p < 0.05$).

The carbonyl content (a) gradually increased on day 10, and decreased significantly after 16 days. This may be because the carbonyl group produced by the oxidation of protein further reacted to form acids or reacted with amino acids to form azomethines. In addition, aldol condensates may also form between the carbonyl molecules.

3.3.2 Sulfhydryl content

Oxidation of proteins causes the destruction of sulfhydryl groups to form disulphide bonds, leading to protein cross-linking [33]. The sulfhydryl group content was inversely proportional to the degree of protein oxidation. Therefore, the loss of sulfhydryl groups is also one of the indicators commonly used to evaluate the degree of protein oxidation.

The sulfhydryl content of sarcoplasmic proteins in sausages decreased during 28 d (Figure 5). After 4 days, the sulfhydryl content of sarcoplasmic protein in the P3-M2 group was significantly higher ($p < 0.05$) than that in the other two groups (Figure 5 a, b). Under oxidising conditions, the internal structure of the sausages was unfolded, and the internal sulfhydryl groups were exposed, resulting in increased sulfhydryl content. The changes in the sulfhydryl content of sarcoplasmic protein and myofibrillar protein showed that the addition of *L. plantarum* P3-M2 could reduce the loss of protein sulfhydryl groups in sausage and the degree of protein oxidation in sausages. This was consistent with the findings of Zhang et al. [34], indicating that adding rat wort extract to sausage as a natural antioxidant can delay the damage of sulfhydryl groups.

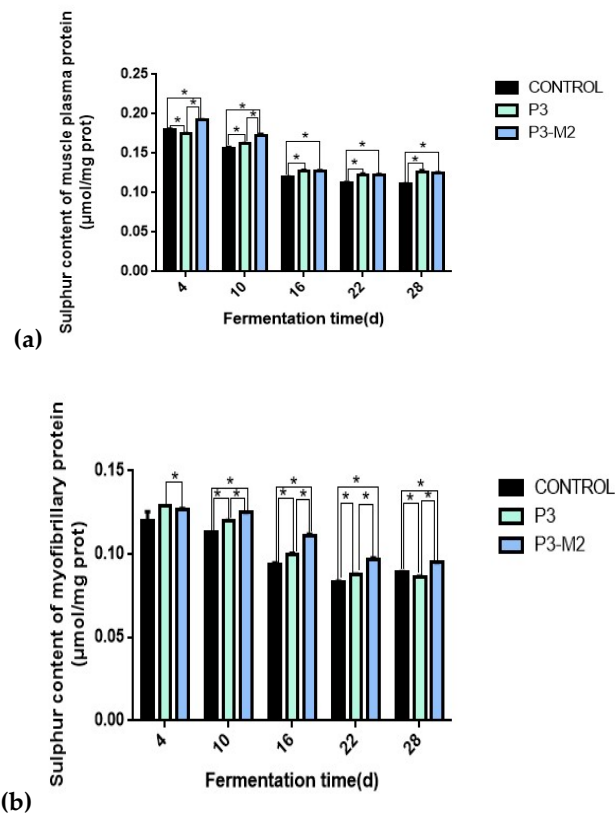


Figure 5. Changes of sulphhydryl group content in sarcoplasmic protein(a) and myofibrillar protein(b) of three groups during fermentation. Data are expressed as the mean of triplicate samples \pm standard error. Vertical bars represent the standard errors of the mean; * above the bars indicate statistically significant differences at $p < 0.05$.

3.3.3. Surface hydrophobicity of myoplasmic protein and myofibrillar protein

The surface hydrophobicity of sarcoplasmic protein was determined using an ANS fluorescent probe. Myofibrillar protein is a salt-soluble protein, so its surface hydrophobicity was determined using the bromophenol blue method.

The changes in the hydrophobicity of the sausages are shown in Figure 6(a, b). It was found that the surface hydrophobicity gradually increased. With the occurrence of protein oxidation and protein degradation, the protein structure was destroyed, and the hydrophobic amino acids inside the protein were exposed, leading to an increase in surface hydrophobicity. The surface hydrophobicity of the control group in muscle plasma protein was higher than that in the P3 and P3-M2 groups, especially after 10 days ($p < 0.05$). The surface hydrophobicity of the P3-M2 group was significantly lower than that of the other two groups ($p < 0.05$), suggesting that strain P3-M2 could inhibit protein oxidation, which reduced the hydrophobic groups from the degree of protein oxidation, and the addition of the strain reduced the surface hydrophobicity of sausage sarcoplasmic protein, and the protein structure was protected to a certain extent. The hydrophobicity of the P3-M2 group was always the lowest, indicating that the mutant strain p3-M2 could slow down the degree of oxidation of sarcoplasmic protein.

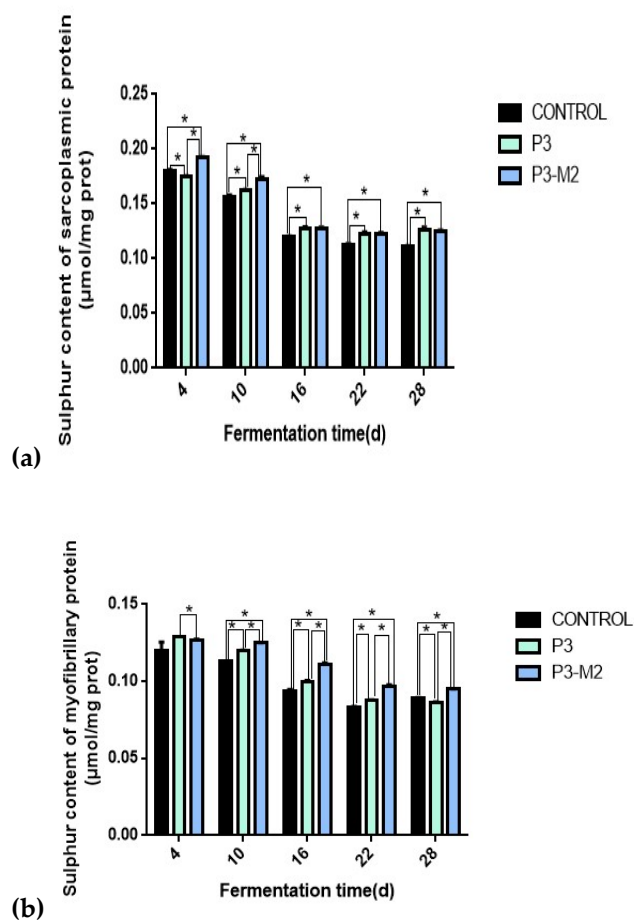


Figure 6. Changes of surface hydrophobicity in sarcoplasmic proteins(a) and myofibrillar protein(b). Different capital letters indicate significant differences in the surface hydrophobicity of sarcoplasmic proteins between the three groups of samples ($p < 0.05$); different lowercase letters indicate significant differences in the surface hydrophobicity of the sarcoplasmic proteins during the same group of fermentation ($p < 0.05$).

3.3.4 α -helix structure changes of myoplasmic protein and myofibrillar protein

The α -helix is mainly a stable hydrogen bond between carbonyl and amino groups generated by the polypeptide chain. Oxidation can lead to a reduced α -helix content.

The α -helix structure of the sausage is shown in Figure 7. The relative content of α -helix showed a tendency to decrease during the fermentation process. Hydrogen bonding between the carbonyl and amino groups is damaged by exposure to the hydrophobic groups of oxidation. Thus, the α -helix structure was reduced by peptide bond breakage in the protein [35].

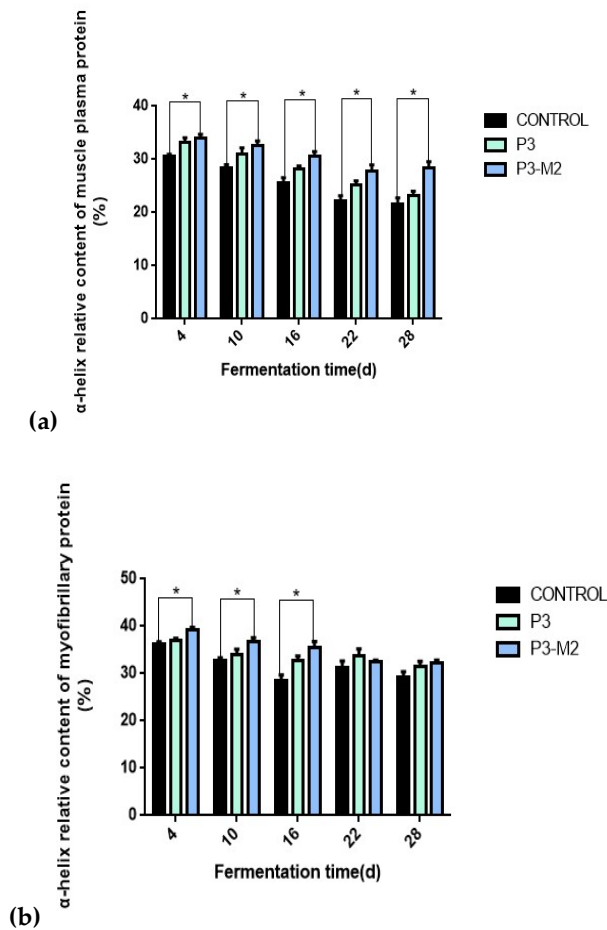


Figure 7. Changes of α -helix relative content in sarcoplasmic protein and myofibrillar protein of three groups during fermentation. Different lowercase letters indicate that the relative content of sarcoplasmic protein α -helix is significantly different among the three groups of samples ($p < 0.05$), and different lowercase letters indicate that the relative content of sarcoplasmic protein α -helix is significantly different during the same group of fermentation ($p < 0.05$).

As the control group of sausage did not add the other ingredients with antioxidant ability, the 4 d α -helix relative content in the control group was lower than that in the other two groups ($p < 0.05$). The relative content of the group P3 α -helix structure was lower than that of the P3-M2 group because the antioxidant activity of the P3-M2 strain was stronger than that of the P3 group.

3.3.5 MetMb content

Myoglobin has the function of depositing pigment and exists inside the cells, making the meat appear red. Myoglobin was oxidised to produce oxygenated myoglobin, making the meat appear bright red. Oxymyoglobin can then be further oxidised to form metmyoglobin, making the meat brown.

On the day 10, the MetMb content (Figure 8) of the P3-M2 group was significantly lower than that of the control group ($p < 0.05$). The overall MetMb content gradually increased. The MetMb content in the control group was always at the highest level, which also explained that the colour of sausages in the control group changed significantly during the fermentation process ($p < 0.05$). *Lactobacillus plantarum* P3-M2 was shown to reduce the production of MetMb and the rate of myoglobin oxidation.

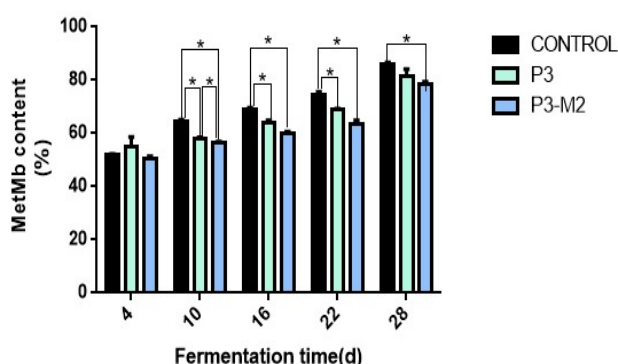


Figure 8. Changes of MetMb content in three groups during fermentation. Data are expressed as the mean of triplicate samples \pm standard error. Vertical bars represent the standard errors of the means. *above the bars indicate statistically significant differences ($p < 0.05$).

3.3.6 SDS-PAGE of myoplasmic protein and myofibrillar protein

Protein hydrolysis generates non-volatile substances such as peptides and free amino acids, which play an important role in the flavour of sausage products [36].

As shown in Figure 9(A), the protein bands of sarcoplasmic protein in fermented sausage were much higher, and the difference in molecular weight was small. HMWP was gradually degraded during fermentation, and the protein band strength of the control group was higher than that of the other two groups on the day 28. P3 and P3-M2 play a role in promoting protein degradation during fermentation. SDS-PAGE results of the three groups of sausage sarcoproteins showed that the protein strips between 98 kDa and 180 kDa gradually disappeared, indicating that the high-molecular weight protein was degraded.

The 25 kDa bands, one between 34 kDa protein bands, gradually disappeared with an extended fermentation time and were speculated to be enzymes or peptides with antioxidant activity. Weizheng Sun [37] also reported that the disappeared proteins were related to antioxidant activity. The control group of sarcoplasmic protein electrophoresis in the protein bands at the day 16 has largely disappeared completely, indicating more severe muscle plasma protein oxidation.

The SDS-PAGE bands of the sausage muscle plasma protein of the P3 group largely disappeared on the day 22, while the electrophoresis of the P3-M2 sausage sarcoplasmic protein showed that the protein band was weakened but existed at the day 28.

The three groups of sausage sarcoplasm protein from 10 to 17 kDa protein bands gradually disappeared. The 14 kDa protein bands may be myoglobin, which can be combined with oxygen and decrease gradually under oxidising conditions. The protein bands between 25 kDa and 34 kDa also gradually disappeared possibly due to creatine kinase.

On the day 22 and 28, the protein bands mainly concentrated between 34 kDa and greater than 17 to 25 kDa. This is because of the degradation of macromolecular proteins and polymerization of small molecular proteins due to the function of endogenous enzymes and enzymes produced by lactic acid bacteria. The large molecular weight protein bands in p3-M2 group were significantly weakened at the day 28, indicating that strain p3-M2 could also promote the hydrolysis of sausage sarcoplasin. With the action of lactic acid bacteria, sarcoplasmic protein will produce hydrophilic polypeptides, the production of hydrophilic polypeptides was conducive to good flavour formation.

As shown in Figure 9(B), the degradation of myofibrillar protein by strains P3 and P3-M2 was not as significant as that by strains P3 and P3-M2. In the electrophoresis diagram of the three groups of sausage myofibrillar protein, the changes in protein bands were mainly between 98 to 180 kDa, 62 kDa, and 25 to 34 kDa. A new protein band

appeared at 62 kDa. The electrophoresis of myofibrillar protein in groups P3 and P3-M2 showed that the high-molecular-weight protein degraded to a low-molecular-weight protein at the day 22 and 28, indicating that strains P3 and P3-M2 had a certain promoting effect on the hydrolysis of myofibrillar protein.

In SDS-PAGE, the degree of hydrolysis of myofibrillar protein and sarcoplasmic protein in the P3-M2 group was higher, which may be due to the small change in protein structure under low oxidation level. However, when proteins are highly oxidised, protein polymers can be formed, and some special amino acid side chains can also be oxidised, leading to reduced protease recognition sites, thus decreasing the degree of protein hydrolysis [38].

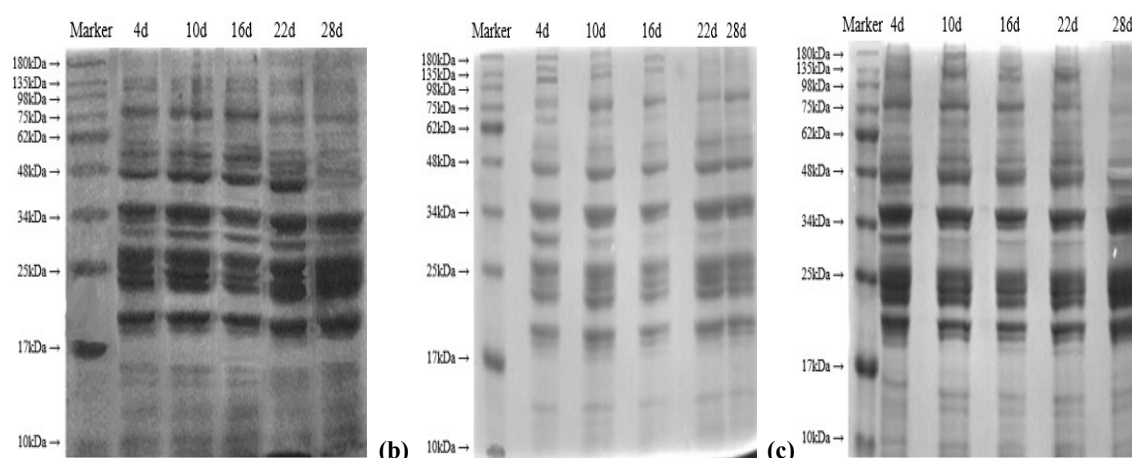


Figure 9(A). SDS-PAGE electrophoresis of sarcoplasmic protein in sausage on the day 4, 10, 16, 22 and 28. (A) (a is CK group, b is P3 group, and c is P3-M2 group).

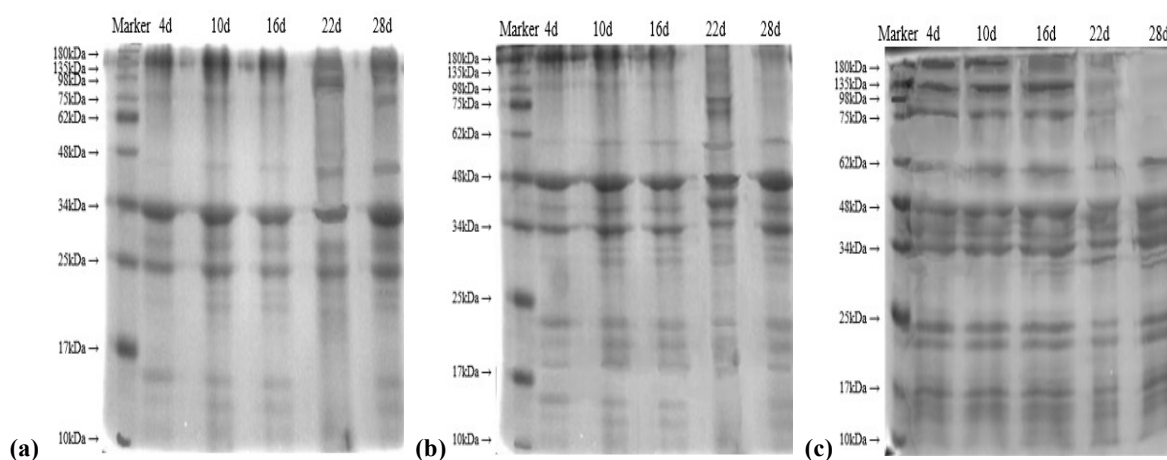


Figure 9(B). SDS-PAGE electrophoresis of myofibrillar protein in sausage (B) on the day 4, 10, 16, 22 and 28. (a is CK group, b is P3 group, and c is P3-M2 group).

3.4 Volatile compounds

The flavour of fermented meat products is caused by the production and accumulation of volatile and non-volatile aromatic compounds and other flavour-related compounds, such as alcohols, aldehydes, ketones, and esters. Proteins, carbohydrates, and lipids are the precursors of aromatic compounds [39]. The oxidation of lipids and proteins in meat products produces ketones, aldehydes, and other substances that directly affect their taste and flavour. Lactic acid bacteria can use carbohydrates, proteins,

organic acids, amino acids, and other nutrients to produce volatile and non-volatile flavour compounds.

The results of volatile substances and their relative percentages are shown in Figure 10 and Table 1. A total of 153 volatile substances were detected by GC-MS. The relative contents of total esters increased by 53.43% and 48.67%. The relative contents of total alcohols decreased by 30.27% and 48.52%. The relative contents of total aldehydes decreased by 8.83% and 32.92%. Total acid decreased 8.98% and 32.67%. Ketones decreased 2.07% and 8.97%. And total terpenes decreased 25.92% and 32.84%.

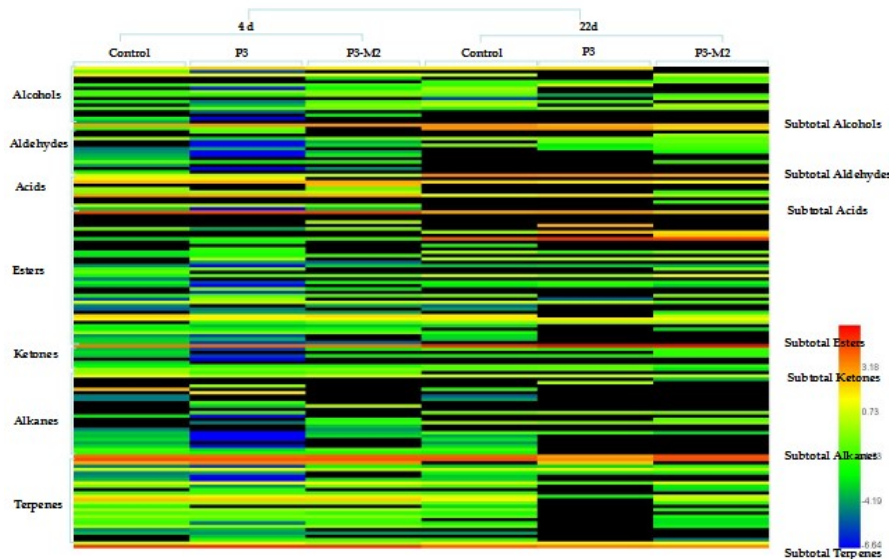


Figure 10. Changes of relative content of volatile substances in control sausage(a), P3(b) and P3-M2(c) on the day 4 and 22.

Table 1. Concentration of volatile compounds in three groups sausages on the day 4 and 22. (%).

RT(min)	Compounds	4d			22d		
		control	P3	P3-M2	control	P3	P3-M2
4.869	Ethyl alcohol	2.69 ± 0.34	4.91 ± 0.51	3.52 ± 1.29	3.57 ± 1.73	4.17 ± 2.33	-
9.589	N-pentanol	0.48 ± 0.03	0.02 ± 0.01	-	-	-	-
10.98	2,3-butanediol	3.74 ± 0.42	2.5 ± 0.15	2.37 ± 1.04	1.34 ± 1.05	-	1.6 ± 1.14
16.738	Cineole	-	-	0.14 ± 0.06	-	-	0.35 ± 0.06
17.414	1-Octen-3-ol	-	-	-	0.32 ± 0.05	0.26 ± 0.01	0.53 ± 0.11
18.967	eucalyptol	0.23 ± 0.07	0.44 ± 0.12	0.34 ± 0.21	0.29 ± 0.06	1.73 ± 1.13	-
19.027	Benzyl alcohol	-	0.01 ± 0.01	0.17 ± 0.09	1.1 ± 0.74	-	-
20.462	Linalool	0.34 ± 0.11	0.78 ± 0.18	0.73 ± 0.25	0.76 ± 0.16	-	-
20.886	phenylethyl alcohol	0.35 ± 0.09	0.26 ± 0.15	0.74 ± 0.04	0.44 ± 0.22	0.07 ± 0.03	0.32 ± 0.12
22.048	2-camphanol	-	-	-	0.02 ± 0.01	-	0.62 ± 0.09
22.061	d-borneol	0.18 ± 0.07	0.04 ± 0.02	0.35 ± 0.08	0.78 ± 0.04	0.37 ± 0.14	-
22.204	4-terpineol	-	0.08 ± 0.04	0.77 ± 0.21	1.65 ± 1.11	-	1.2 ± 1.13
22.432	alpha terpineol	0.35 ± 0.12	0.56 ± 0.10	0.55 ± 0.09	0.21 ± 0.14	0.7 ± 0.03	0.76 ± 0.07

25.409	1-Tetradecanol	-	0.03 ± 0.01	-	-	-	-
28.003	1-Tridecanol	-	-	0.15 ± 0.07	-	-	-
28.005	1-Hexadecanol	0.17 ± 0.21	0.01 ± 0.01	-	-	-	-
29.361	6-Apigenin-4-ol	0.06 ± 0.04	-	-	-	-	-
	Subtotals (%)	8.69 ± 3.50A	9.65 ± 2.31A	9.84 ± 2.15A	10.47 ± 1.50A	7.3 ± 2.14A	5.39 ± 1.10B
11.291	hexanal	0.32 ± 0.05	0.61 ± 0.10	-	9.56 ± 3.16	8.84 ± 2.23	4.4 ± 2.17
16.73	2-Heptenal	-	0.44 ± 0.12	-	-	-	-
16.989	(E)-2-heptenal	-	-	-	-	-	0.92 ± 0.23
18.062	benzaldehyde	0.95 ± 0.07	0.13 ± 0.05	0.6 ± 0.14	1.05 ± 0.10	0.65 ± 0.21	0.48 ± 0.09
19.242	n-octanal	-	0.01 ± 0.03	0.06 ± 0.03	-	0.53 ± 0.07	0.53 ± 0.11
19.494	phenylacetaldehyde	-	0.01 ± 0.01	0.13 ± 0.06	0.72 ± 0.18	0.14 ± 0.03	0.38 ± 0.14
21.682	trans-2-octenal	0.04 ± 0.02	0.07 ± 0.05	-	-	0.17 ± 0.11	0.33 ± 0.09
23.507	trans-2-nonanal	0.05 ± 0.04	0.01 ± 0.01	0.07 ± 0.05	-	-	0.2 ± 0.09
23.569	Trans-2-decene aldehyde	0.07 ± 0.05	0.01 ± 0.01	0.19 ± 0.03	-	-	-
23.841	p-tolualdehyde	0.09 ± 0.07	-	-	-	-	-
25.183	trans-cinnamaldehyde	0.49 ± 0.14	0.17 ± 0.09	0.26 ± 0.08	-	-	0.37 ± 0.15
30.586	γ-nonalactone	0.06 ± 0.04	-	-	-	-	-
30.585	pentadecanal	-	0.01 ± 0.04	0.05 ± 0.02	-	-	-
11.291	hexadecanal	0.18 ± 0.08	-	-	-	-	-
	Subtotals (%)	2.25 ± 1.22A	1.45 ± 0.94A	1.37 ± 1.43A	11.33 ± 2.98A	10.33 ± 2.52A	7.60 ± 2.31A
6.522	oxalic acid	3.46±1.13	3.55±1.32	-	-	-	-
10.048	acetic acid	6.28 ±1.47A	7.15 ± 1.37A	7.32 ± 0.21A	4.63 ± 0.95A	3.83 ± 0.48A	3.1 ± 0.09B
10.891	3-methyl pentanoic acid	-	-	6.36 ± 2.56	-	-	-
11.372	3-methyl butyric acid	0.97 ± 0.07	0	0.53 ± 0.32	-	-	-
11.455	2-methyl butyric acid	0.77±0.09	1.08±0.21	1.39 ± 0.16	-	-	0.27 ± 0.16
13.124	n-caproic acid	8.27 ± 2.43	7.06 ± 2.27	6.56 ± 1.15	2.39 ± 1.25	3.81 ± 1.27	1.07 ± 0.96
14.676	(s)-(+)-malic acid	-	-	-	-	-	0.28 ± 0.04
17.913	caprylic acid	1.19 ± 0.18	1.29 ± 0.37	0.52 ± 0.11	-	-	-
19.031	3-methylbut-2-en oic acid	0.09±0.04	0.01±0.02	0.08±0.05	-	-	-
19.49	Subtotals (%)	21.02 ± 3.41A	20.14 ± 2.97A	22.75 ± 2.53A	7.01 ± 3.37A	7.64 ± 2.51A	4.72 ± 2.78A
19.758	ethyl isobutyrate	-	-	1.22 ± 1.02	-	-	-
20.327	ethyl lactate	-	-	-	-	6.7 ± 2.35	-
20.42	Cinnamic acid	0.52 ± 0.32	0.07 ± 0.05	0.66 ± 0.29	-	-	-

	vinylester						
20.46	Ethyl 3-methylbutyrate	-	-	-	0.97 ± 0.10	6.46 ± 2.44	4.16 ± 1.01
20.683	Benzoic acid-2-phenylethyl ester	-	-	-	-	-	5.47 ± 0.32
21.986	ethyl hexanoate	0.13 ± 0.06A	0.28 ± 0.09A	0.35 ± 0.06B	16.57 ± 2.77A	27.41 ± 3.25B	26.48 ± 3.86B
22.188	Ethyl DL-Ma0elate	-	0.17 ± 0.07	-	-	-	-
22.212	Decyl methacrylate	-	-	-	0.22 ± 0.06	-	-
22.291	octyl formate	-	0.29 ± 0.11	-	-	-	-
22.91	ethyl heptylate	0.14 ± 0.09	0.26 ± 0.15	-	0.85 ± 0.17	0.29 ± 0.11	0.59 ± 0.13
23.018	3-methylbutyl-2-methylbutyrate	0.13 ± 0.07	0.2 ± 0.13	0.33 ± 0.13	-	-	-
23.228	linalyl formate	-	1.25 ± 0.15	-	1.05 ± 0.9	1.22 ± 0.15	1.59 ± 0.43
23.27	Butane-2-yl-3-methylbutyrate	-	0.06 ± 0.03	0.03 ± 0.01	-	-	-
23.959	ethyl benzoate	0.05 ± 0.04	0.01 ± 0.01	0.1 ± 0.06	0.09 ± 0.04	0.1 ± 0.05	0.07 ± 0.05
24.529	butyl caproate	0.33 ± 0.19	0.6 ± 0.24	-	-	-	0.6 ± 0.32
24.53	butyric acid hexylester	0.57 ± 0.25	-	-	-	-	-
24.951	ethyl ocanoate	0.19 ± 0.07	0.43 ± 0.13	0.53 ± 0.32	1.56 ± 0.23	0.91 ± 0.14	2.41 ± 1.45
24.977	2-ethylhexyl acrylate	0.03 ± 0.03	-	-	-	-	-
25.181	hexyl isovalerate	0.19 ± 0.13	0.02 ± 0.01	0.2 ± 0.11	0.21 ± 0.09	0.3 ± 0.16	0.2 ± 0.09
25.317	isoamyl caproate	0.09 ± 0.04	0.01 ± 0.01	-	0.14 ± 0.07	0.23 ± 0.14	0.1 ± 0.07
25.339	Hexanoic acid,2-methylbutyl ester	-	0.8 ± 0.37	0.15 ± 0.08	-	-	-
25.432	n-decyl formate	-	0.02 ± 0.01	-	-	-	-
26.055	ethylnonanoate	0.22 ± 0.10	0.45 ± 0.21	0.39 ± 0.12	0.63 ± 0.24	-	0.72 ± 0.32
26.626	2-Butenoic acid,2-methyl-,2-methylpropyl ester,(2E)-(E)-hexyl	0.02 ± 0.01	0.92 ± 0.06	-	-	0.03 ± 0.03	-
27.886	2-Methylbut-2-en oate	1.58 ± 0.97	1.41 ± 0.99	1.28 ± 0.26	1.63 ± 0.16	1.3 ± 0.09	0.36 ± 0.11
29.318	Ethyl3-phenylpropionate	0.04 ± 0.03	-	0.06 ± 0.03	0.03 ± 0.02	-	-

30.272	terpinyl acetate	0.03 ± 0.03	0.04 ± 0.02	-	0.06 ± 0.04	-	-
32.303	4-hydroxydecanoic acid	-	0.06 ± 0.04	0.06 ± 0.01	-	-	0.21 ± 0.10
6.522	hexyl caproate	4.47 ± 1.98	3.36 ± 1.84	4.41 ± 1.07	3.57 ± 1.28	-	1.46 ± 0.16
10.048	n-butyl octanoate	3.33 ± 1.39	3.25 ± 1.99	2.98 ± 1.37	3.64 ± 1.32	3.79 ± 1.11	2.8 ± 0.98
10.891	ethyl caprate	-	0.23 ± 0.16	0.42 ± 0.18	0.72 ± 0.21	1.07 ± 0.05	0.92 ± 0.35
11.372	2-Hexenoic acid, butyl ester, (2E)-	0.17 ± 0.09	0.08 ± 0.04	0.09 ± 0.05	0.11 ± 0.03	-	-
11.455	ethyl cinnamate	0.16 ± 0.07	0.16 ± 0.08	0.2 ± 0.13	-	-	0.14 ± 0.06
13.124	hexyl octanoate	0.93 ± 0.21	0.73 ± 0.31	0.52 ± 0.17	0.22 ± 0.06	-	-
14.676	(Z)-dihydro-5-(2-octenyl)furan-2(3H)-one	0.05 ± 0.03	0.02 ± 0.02	-	0.11 ± 0.10	-	-
17.913	Ethyl hydride myristate	0.1 ± 0.09	0.08 ± 0.04	-	0.1 ± 0.04	-	-
19.031	ethyl palmitate	0.11 ± 0.03	0.02 ± 0.02	0.03 ± 0.02	-	-	-
	Subtotals (%)	13.58 ± 3.43A	15.28 ± 2.45A	14.03 ± 3.02A	32.47 ± 3.11A	49.82 ± 3.90B	48.27 ± 4.24B
17.607	6-Methyl-5-hepten-2-one	0.08 ± 0.06	0.02 ± 0.02	0.16 ± 0.10	0.36 ± 0.12	0.22 ± 0.16	0.33 ± 0.14
19.817	acetophenone	0.12 ± 0.07	-	-	-	-	0.29 ± 0.12
20.237	2-nonanone	0.09 ± 0.04	0.02 ± 0.02	0.19 ± 0.03	0.21 ± 0.14	0.22 ± 0.97	0.29 ± 0.16
23.981	2-U0ecanone	-	0.01 ± 0.01	-	-	-	-
23.99	2-u0ecanone	0.11 ± 0.07	-	-	-	-	-
24.417	2-isopropyl-5-methyl-3-cyclohexen-1-one	0.16 ± 0.13	0.25 ± 0.08	0.24 ± 0.12	0.43 ± 0.07	0.46 ± 0.09	0.28 ± 0.07
27.08	Apitanedienone	0.9 ± 0.11	0.63 ± 0.15	0.73 ± 0.09	0.45 ± 0.11	0.52 ± 0.07	0.13 ± 0.02
30.995	(+)-Cyperone	0.88 ± 0.22	0.32 ± 0.07	0.41 ± 0.03	-	-	-
	Subtotals (%)	2.34 ± 1.75A	1.25 ± 0.69A	1.73 ± 0.89A	1.45 ± 0.13A	1.42 ± 0.67A	1.32 ± 0.95A
3.527	Diethanolic anhydride	-	-	-	-	-	0.08 ± 0.06
4.704	ammonium carbamate	-	-	-	-	1.06 ± 0.21	-
4.808	formamide	-	0.85 ± 0.15	-	-	-	-
6.321	methoxyacetic anhydride	5.99 ± 2.46	-	-	0.27 ± 0.11	-	-
6.478	ammonium acetate	-	3.86 ± 1.78	-	-	-	-
9.721	Methanesulfonic anhydride	0.04 ± 0.03	-	-	0.03 ± 0.02	-	-
13.016	formhydrazide	0.04 ± 0.04	-	-	0.06 ± 0.04	-	-

13.145	valerate anhydride	-	0.36 ± 0.12	-	-	-	-
15.261	2-Oxime-2-methoxy- phenyl	-	0.1 ± 0.07	1.35 ± 0.19	-	-	-
15.331	2-Methylpentanoic anhydride	-	0.37 ± 0.21	-	0.61 ± 0.28	0.92 ± 0.14	0.69 ± 0.12
17.488	n-Hexanoic anhydride	0.14 ± 0.08	0.01 ± 0.01	-	-	-	-
17.52	2,3,5-trimethylpyrazine	-	-	0.23 ± 0.13	-	-	0.42 ± 0.13
18.104	butylbenzene	-	0.04 ± 0.02	0.32 ± 0.17	0.95 ± 0.32	0.68 ± 0.25	0.78 ± 0.27
18.76	pentylcyclopropane	-	-	0.09 ± 0.04	-	-	-
19.49	2-ethyl-3,6-dimethylpyrazine	0.04 ± 0.01	-	0.05 ± 0.04	-	-	-
20.03	ligustrazine	0.09 ± 0.03	0.01 ± 0.01	0.08 ± 0.04	0.38 ± 0.14	0.29 ± 0.05	0.11 ± 0.08
20.201	1-ethenyl-3,5-dimethyl-benzene	0.08 ± 0.04	0.01 ± 0.01	0.12 ± 0.07	0.06 ± 0.04	-	-
20.33	n-tridecane	0.12 ± 0.09	0.01 ± 0.01	-	0.14 ± 0.10	-	-
24.036	2-methylnaphthalene	0.06 ± 0.02	-	-	0.05 ± 0.02	-	-
24.659	7-methylheptadecane	0.11 ± 0.07	0.01 ± 0.01	-	0.12 ± 0.06	-	-
25.095	Cyclotetradecane	0.35 ± 0.13	0.11 ± 0.07	0.39 ± 0.13	0.38 ± 0.13	-	-
25.404	n-tetradecane	0.29 ± 0.21	0.21 ± 0.12	0.22 ± 0.07	0.18 ± 0.05	-	-
25.506	anethole	15.65 ± 1.74	14.98 ± 1.99	17.03 ± 1.06	17.26 ± 2.21	8.11 ± 1.87	19.34 ± 1.14
	Subtotals (%)	22.99 ± 3.76A	20.3 ± 2.78A	19.96 ± 2.54A	20.49 ± 3.08A	11.07 ± 2.33B	21.43 ± 2.07A
14.665	styrene	7.03 ± 3.22	10.44 ± 3.01	9.9 ± 3.00	-	5.34 ± 3.02	-
18.234	phellandrene	0.04 ± 0.02	0.02 ± 0.01	0.18 ± 0.07	0.11 ± 0.07	0.11 ± 0.09	0.28 ± 0.04
18.552	(+)-Limonene	1.7 ± 0.14	2.27 ± 1.34	1.38 ± 0.22	1.92 ± 0.14	2.66 ± 0.53	1.34 ± 1.07
18.56	γ-terpinolene	0.04 ± 0.03	0.06 ± 0.03	-	0.16 ± 0.09	0.14 ± 0.04	0.16 ± 0.07
23.355	ocimene	0.05 ± 0.04	0.01 ± 0.01	-	0.09 ± 0.03	-	-
25.475	(-)-α-linolenene	0.08 ± 0.03	0.01 ± 0.01	0.14 ± 0.10	0.11 ± 0.01	-	-
25.54	α-copaene	1.83 ± 0.77	2.49 ± 1.08	2.89 ± 0.97	1.96 ± 0.97	0.38 ± 0.55	2.05 ± 1.12
25.705	beta-elemene	0.64 ± 0.11	0.05 ± 0.05	0.56 ± 0.01	0.36 ± 0.11	-	0.14 ± 0.08
26.249	6-methyl-2-(4-methylcyclohex-3-enyl)hept-2,5-diene	-	0.29 ± 0.08	-	-	-	0.12 ± 0.05
26.252	β-caryophyllene	0.3 ± 0.08	0.31 ± 0.11	0.32 ± 0.02	0.26 ± 0.03	-	-
26.846	Alpha-curcumene	3.8 ± 1.32	3.35 ± 1.56	3.75 ± 0.14	2.11 ± 1.05	0.18 ± 0.06	1.64 ± 0.15

26.912	valencene	6.04 ± 3.02	5.37 ± 2.09	5.13 ± 3.01	3.42 ± 0.95	-	1.36 ± 0.94
26.998	zingiberene	1.18 ± 0.18	1.1 ± 0.19	1.04 ± 0.24	0.5 ± 0.17	-	0.27 ± 0.10
27.042	α-farnesene	0.22 ± 0.16	-	-	0.11 ± 0.07	-	0
27.259	β-selinene	1.33 ± 0.97	0.9 ± 0.14	1.11 ± 0.17	0.67 ± 0.01	-	0.24 ± 0.04
27.305	cis-linalool-4(14),5-diene	0.33 ± 0.04	0.42 ± 0.18	0.25 ± 0.04	0.2 ± 0.13	-	0
27.407	β-sesquiphellandrene	0.96 ± 0.16	0.75 ± 0.21	0.84 ± 0.14	0.43 ± 0.16	-	0.21 ± 0.07
27.477	(+)-d-butylene	0.41 ± 0.08	0.33 ± 0.10	0.36 ± 0.09	0	-	0.12 ± 0.09
27.525	(-)-cis-calamenene	0.31 ± 0.10	0.03 ± 0.03	0.29 ± 0.12	0.21 ± 0.07	-	0.12 ± 0.14
27.567	α-panasinsene	1.23 ± 0.13	0.75 ± 0.04	0.91 ± 0.25	0.53 ± 0.08	-	0.16 ± 0.07
27.652	(-)-Alpha-cubebene	0.04 ± 0.03	0.07 ± 0.04	-	-	-	-
27.813	α-calacorene	0.08 ± 0.03	0.05 ± 0.04	0.06 ± 0.04	0.06 ± 0.04	-	-
28.001	1-Tricene	-	0.09 ± 0.05	-	-	-	-
29.356	γ-selinene	-	0.27 ± 0.09	0.35 ± 0.01	0.24 ± 0.13	-	0.04 ± 0.04
22.514	4-Allylanisole	1.49 ± 0.14	2.51 ± 1.27	1.21 ± 0.14	3.36 ± 1.04	3.63 ± 1.03	3.02 ± 0.96
Subtotals (%)		29.12 ± 3.38A	31.93 ± 3.79A	30.68 ± 4.90A	16.78 ± 2.21A	12.43 ± 1.32B	11.27 ± 1.17B

The contents of the volatile compounds were expressed as mean ± standard deviation (n = 3). RI, retention index; Hyphens in content columns mean not detected. Different letters (A–C) in the same row indicate significant differences among the three groups in the same days. (*p* < 0.05).

Esters with a lower threshold are formed by the esterification reaction of acids and alcohols. The esters formed by short-chain acids have a fruity flavour, while long-chain acids have a greasy taste, which helps produce the special flavour of fermented sausages. Thirty-nine types of esters were detected, including ethyl caproate with an aroma of koji and pineapple, hexyl hexanoate with a fragrant fruit flavour, butyl caprylate with a buttery smell, linalyl formate with an aroma of fruit and rose, and hexyl tiglate which is a synthetic fragrance. As shown in Figure 10, the relative percentage of esters increased with the extension of fermentation time, so flavours gradually accumulated. It also can be found that the relative percentage of esters of P3 and P3-M2 groups were higher than the control group (*p* < 0.05) after 22 days. In particular, Ethyl 3-methylbutyrate increased by 566% and 329%, acid ethyl ester by 39.82%, ethyl hexanoate by 65.42% and 59.81%, linalyl formate by 16.19% and 51.43%. This can be explained by the fact that strains P3-M2 and P3 helped in the generation of flavour(*p* < 0.05). However, there was no significant difference between P3 and P3-M2 groups (*p* ≥ 0.05).

A total of 10 types of acids were detected, mainly acetic and hexanoic acids. Acids can directly affect the flavour of sausage and form esters with alcohols. The acid content was high in the initial sausage fermentation process and decreased with the generation and reduction of alcohols. Acids are the main components of fermented sausage flavour substances [5], such as N-hexanoic acid with a flavour similar to dry cheese, are the main components of fermented sausage flavour [5]. On the day 22, acetic acid in P3-M2 groups were significantly lower than those in the control group(*p* < 0.05).The alcohols and organic acids from the metabolism of lactic acid bacteria could generate the corresponding ester under the action of esterase (ES) and alkyltransferase (AAT).

Alcohols are mainly produced by the metabolism of carbohydrates under the action of microorganisms, and some alcohols can also be produced by lipid oxidation. A total of 17 types of alcohols were detected in the three sausage groups, mainly ethanol with a special aroma and slight irritation. The 1-octene-3-alcohol was a strong mushroom-like sweet herb and dry grass-like soil fragrance, 2, 3-butanediol showed a gentle rise and honey-like aroma. From Figure 11, it can be observed that the relative percentage of alcohols showed a trend of rising first and then falling. The increase in the early stage was mainly due to the metabolism of sugars to produce alcohols with the action of microorganisms, while in the later stage, these alcohols could undergo an esterification reaction with acids to form esters, resulting in a decrease in their relative percentage. After the day 22, the alcohols in P3-M2 groups were significantly lower than those in the control group and P3 group ($p < 0.05$).

Aldehydes, which are key components of volatile substances in fermented meat products with a low threshold and a strong odour, are mainly derived from the oxidation of lipids and degradation of amino acids. Nonanal and octanal are produced when unsaturated fatty acids are oxidised. Fourteen types of aldehyde were detected. Benzaldehyde and phenylacetaldehyde, which are produced by the degradation of phenylalanine by Strecker, are related to protein oxidation [40], similar to fruity aromas. Roldan et al. [41] also found that the content of hexanal in cooked mutton was significantly related to TBARS, possibly due to the free radicals produced by the rapid oxidation of lipids. In our study, the relative percentages of benzaldehyde and phenylacetaldehyde showed an upward trend. Moreover, the relative percentages of benzaldehyde and phenylacetaldehyde in the P3 and P3-M2 groups was lower than that in the control group, confirming that the carbonyl group was gradually formed during fermentation, and *L. plantarum* P3 could slow down the generation of carbonyl groups ($p < 0.05$).

Most of the ketones have a milky or fruity flavour, with a lower threshold, which is an important component of the special flavour of sausages. For example, the relatively high content of 3-hydroxy-2-butanone, which has a buttery taste, plays an important role in the special flavour of fermented sausages. The ketone content in the three sausage groups was less and with no significant difference ($p \geq 0.05$).

Alkenyl terpenoids are mainly derived from these species. From Figure 10, it was found that the total relative percentage of alkenyl terpenoids in the P3 and P3-M2 groups was higher than that in the control group ($p < 0.05$). This may be because the addition of *lactic acid bacteria* changes the environment of fermentation and promotes the volatilization of flavour in spices.

Based on these results, it can be considered that *L. plantarum* strains with antioxidant ability may affect flavour through protein and lipid oxidation pathways. In the process of amino acid metabolism of LAB, amino acids mainly pass through four metabolic pathways to produce volatile aroma components (Figure 11(a)). One of the amino acid pathways was associated with two kinds of dehydrogenase, α -ketate dehydrogenase and hydroxy acid dehydrogenase. Through these ways, the LAB strains with antioxidant ability may enhance the degradation of amino acid, and promote the aldehyde to produce more alcohols through reduction reaction. Thus from this way, more esters were formed by the reaction of alcohol and acid with the esterase.

Additionally free fatty acids would be produced under the action of esterase, and fatty acids can be further oxidised to produce flavour substances, such as short-chain fatty acids and aliphatic aldehydes (Figure 11(b)). Then the saturated, unsaturated fatty acids and some of the fatty acids were oxidised or β -oxidated to inform alcohol and acid. In our study, it was confirmed that strains P3 and P3-M2 can reduce the oxidation of free fatty acid by inhibiting LOX activity. By the lipid pathway, alcohol and acid were decreased while the ester was increased.

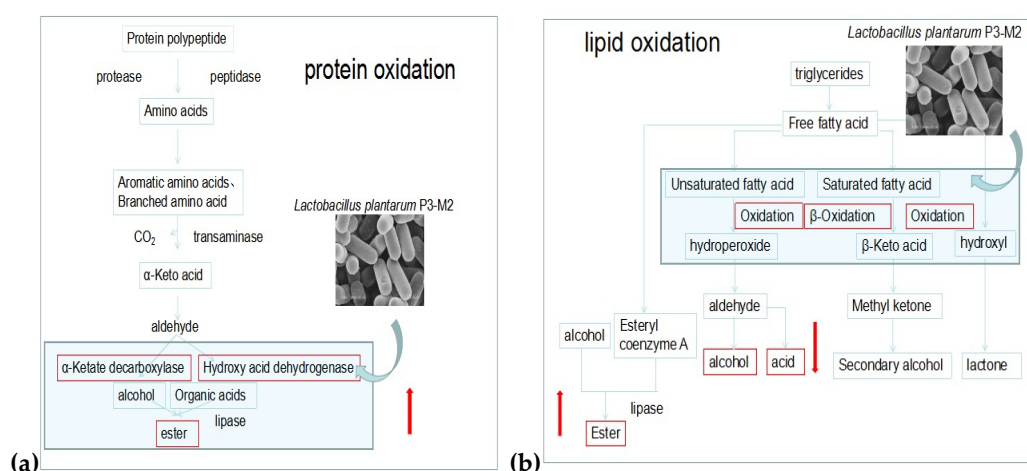


Figure 11. Primarily metabolic pathways of protein (a) and lipid (b) associated with flavour formation in LAB

5. Conclusions

Our results indicated that *L. plantarum* strains P3-M2 and P3 with high antioxidant properties enhance the flavour of Chinese sausages through the reduction of lipid and protein oxidation. The retardation of the oxidation of lipids and proteins in Chinese sausages during storage may be attributed to the antioxidant ability of *L. plantarum*, which changes the alleviation of flavour substances, such as inducing ester and reducing some kinds of alcohol and acid. This study provides valuable information to enhance the flavour of Chinese fermented sausages with the antioxidant *L. plantarum* strain. However, further studies are needed to support new evidence regarding the other mechanisms of the sausage flavour of *L. plantarum* with anti-oxidization ability.

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References

1. Xiao, Y.; Liu, Y.; Chen, C.; Xie, T.; Li, P. Effect of *Lactobacillus plantarum* and *Staphylococcus xylosus* on flavour development and bacterial communities in Chinese dry fermented sausages. *Food Research International* **2020**, *135*, 109247, doi:<https://doi.org/10.1016/j.foodres.2020.109247>.
2. Bartkiene, E.; Bartkevics, V.; Mozurienė, E.; Lele, V.; Zadeike, D.; Juodeikiene, G. The Safety, Technological, Nutritional, and Sensory Challenges Associated With Lacto-Fermentation of Meat and Meat Products by Using Pure Lactic Acid Bacteria Strains and Plant-Lactic Acid Bacteria Bioproducts. *Front. Microbiol.* **2019**, *10*, 4, doi:10.3389/fmicb.2019.01036.
3. Bancalari, E.; Montanari, C.; Levante, A.; Alinovi, M.; Neviani, E.; Gardini, F.; Gatti, M. *Lactobacillus paracasei* 4341 as adjunct culture to enhance flavor in short ripened Caciotta-type cheese. *Food Research International* **2020**, *135*, 109284, doi:<https://doi.org/10.1016/j.foodres.2020.109284>.
4. Zheng, Y.; Fei, Y.; Yang, Y.; Jin, Z.; Yu, B.; Li, L. A potential flavor culture: *Lactobacillus harbinensis* M1 improves the organoleptic quality of fermented soymilk by high production of 2,3-butanedione and acetoin. *Food Microbiology* **2020**, *91*, 103540, doi:<https://doi.org/10.1016/j.fm.2020.103540>.

5. Hu, Y.; Zhang, L.; Liu, Q.; Wang, Y.; Chen, Q.; Kong, B. The potential correlation between bacterial diversity and the characteristic volatile flavour of traditional dry sausages from Northeast China. *Food Microbiology* **2020**, *91*, 103505, doi:<https://doi.org/10.1016/j.fm.2020.103505>.
6. Li, R.-Y.; Zheng, X.-W.; Zhang, X.; Yan, Z.; Wang, X.-Y.; Han, B.-Z. Characterization of bacteria and yeasts isolated from traditional fermentation starter (Fen-Daqu) through a ¹H NMR-based metabolomics approach. *Food Microbiology* **2018**, *76*, 11-20, doi:<https://doi.org/10.1016/j.fm.2018.03.015>.
7. Feng, X.; Tjia, J.Y.Y.; Zhou, Y.; Liu, Q.; Fu, C.; Yang, H. Effects of tocopherol nanoemulsion addition on fish sausage properties and fatty acid oxidation. *LWT* **2020**, *118*, 108737, doi:<https://doi.org/10.1016/j.lwt.2019.108737>.
8. Cheng, J.; Xiang, R.; Tang, D.; Zhu, M.; Liu, X. Regulation of protein oxidation in Cantonese sausages by rutin, quercetin and caffeic acid. *Meat Science* **2021**, *175*, 108422, doi:<https://doi.org/10.1016/j.meatsci.2020.108422>.
9. Jiang, Y.; Li, D.; Tu, J.; Zhong, Y.; Zhang, D.; Wang, Z.; Tao, X. Mechanisms of change in gel water-holding capacity of myofibrillar proteins affected by lipid oxidation: The role of protein unfolding and cross-linking. *Food Chemistry* **2021**, *344*, 128587, doi:<https://doi.org/10.1016/j.foodchem.2020.128587>.
10. Domínguez, R.; Pateiro, M.; Gagaoua, M.; Barba, F.J.; Zhang, W.; Lorenzo, J.M. A Comprehensive Review on Lipid Oxidation in Meat and Meat Products. *Antioxidants* **2019**, *8*.
11. Papuc, C.; Goran, G.V.; Predescu, C.N.; Nicorescu, V. Mechanisms of Oxidative Processes in Meat and Toxicity Induced by Postprandial Degradation Products: A Review. *Compr. Rev. Food. Sci. Food Saf.* **2017**, *16*, 96-123, doi:10.1111/1541-4337.12241.
12. Smaoui, S.; Hsouna, A.B.; Lahmar, A.; Ennouri, K.; Mtibaa-Chakchouk, A.; Sellem, I.; Najah, S.; Bouaziz, M.; Mellouli, L. Bio-preservative effect of the essential oil of the endemic *Mentha piperita* used alone and in combination with BacTN635 in stored minced beef meat. *Meat Science* **2016**, *117*, 196-204, doi:<https://doi.org/10.1016/j.meatsci.2016.03.006>.
13. Zhang, D.; Li, H.; Wang, Z.; Emara, A.M.; Hu, Y.; He, Z. Effects of in vitro oxidation on myofibrillar protein charge, aggregation, and structural characteristics. *Food Chemistry* **2020**, *332*, 127396, doi:<https://doi.org/10.1016/j.foodchem.2020.127396>.
14. Ge, Q.; Chen, S.; Liu, R.; Chen, L.; Yang, B.; Yu, H.; Wu, M.; Zhang, W.; Zhou, G. Effects of *Lactobacillus plantarum* NJAU-01 on the protein oxidation of fermented sausage. *Food Chemistry* **2019**, *295*, 361-367, doi:<https://doi.org/10.1016/j.foodchem.2019.05.154>.
15. Noureen, S.; Riaz, A.; Arshad, M.; Arshad, N. In vitro selection and in vivo confirmation of the antioxidant ability of *Lactobacillus brevis* MG000874. *J. Appl. Microbiol.* **2019**, *126*, 1221-1232, doi:10.1111/jam.14189.
16. Van Ba, H.; Seo, H.-W.; Cho, S.-H.; Kim, Y.-S.; Kim, J.-H.; Ham, J.-S.; Park, B.Y.; Pil Nam, S. Antioxidant and anti-foodborne bacteria activities of shiitake by-product extract in fermented sausages. *Food Control* **2016**, *70*, 201-209, doi:<https://doi.org/10.1016/j.foodcont.2016.05.053>.
17. Lin, M.Y.; Yen, C.L. Inhibition of lipid peroxidation by *Lactobacillus acidophilus* and *Bifidobacterium longum*. *J Agric Food Chem* **1999**, *47*, 3661-3664.
18. Liang, X.L.; Wang, X.L.; Li, Z.; Hao, Q.H.; Wang, S.Y. Improved in Vitro Assays of Superoxide Anion and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical-Scavenging Activity of Isoflavones and Isoflavone Metabolites. *J. Agric. Food Chem.* **2010**, *58*, 11548-11552, doi:10.1021/jf102372t.
19. Lim, H.J.; Kim, G.D.; Jung, E.Y.; Seo, H.W.; Joo, S.T.; Jin, S.K.; Yang, H.S. Effect of Curing Time on the Physicochemical and Sensory Properties of Beef Jerky Replaced Salt with Soy Sauce, Red Pepper Paste and Soybean Paste. *Asian Australas. J. Anim. Sci.* **2014**, *27*, 1174-1180, doi:10.5713/ajas.2013.13853.
20. Witte, V.C.; Krause, G.F.; Bailey, M.E. A new extraction method for determining 2-thiobarbituric acid values for pork and beef during storage. *Journal of Food Science* **1970**, *35*, 585-592.

21. Liu, Y.; Li, J.W.; Cheng, Y.J.; Liu, Y.F. Effect of frying oils' fatty acid profile on quality, free radical and volatiles over deep-frying process: A comparative study using chemometrics. *LWT-Food Sci. Technol.* **2019**, *101*, 331-341, doi:10.1016/j.lwt.2018.11.033.
22. Yu, D.; Feng, M.-q.; Sun, J.; Xu, X.-l.; Zhou, G.-h. Protein degradation and peptide formation with antioxidant activity in pork protein extracts inoculated with *Lactobacillus plantarum* and *Staphylococcus simulans*. *Meat Science* **2020**, *160*, 107958, doi:<https://doi.org/10.1016/j.meatsci.2019.107958>.
23. Soglia, F.; Petracci, M.; Ertbjerg, P. Novel DNPH-based method for determination of protein carbonylation in muscle and meat. *Food Chemistry* **2016**, *197*, 670-675, doi:<https://doi.org/10.1016/j.foodchem.2015.11.038>.
24. Wang, Z.; He, Z.; Gan, X.; Li, H. Effect of peroxy radicals on the structure and gel properties of isolated rabbit meat myofibrillar proteins. *International Journal of Food Science and Technology* **2018**, *53*, 2687-2696, doi:10.1111/ijfs.13878.
25. Smaoui, S.; Ennouri, K.; Chakchouk-Mtibaa, A.; Karray-Rebai, I.; Hmidi, M.; Bouchaala, K.; Mellouli, L. Relationships Between Textural Modifications, Lipid and Protein Oxidation and Sensory Attributes of Refrigerated Turkey Meat Sausage Treated with Bacteriocin BacTN635. *Food and Bioprocess Technology* **2017**, *10*, 1655-1667, doi:10.1007/s11947-017-1933-0.
26. Chen, Q.; Kong, B.; Han, Q.; Liu, Q.; Xu, L. The role of bacterial fermentation in the hydrolysis and oxidation of sarcoplasmic and myofibrillar proteins in Harbin dry sausages. *Meat Science* **2016**, *121*, 196-206, doi:<https://doi.org/10.1016/j.meatsci.2016.06.012>.
27. Song, Y.; Qiao, N.; Li, C.W.; Wen, T.T.; Liu, F.Y. SPME-GC/MS Analysis of Volatile Components from Air-dried Sausage during Processing. *Advanced Materials Research* **2013**, *781-784*, 1614-1618.
28. Latoch, A.; Libera, J. Quality and Safety of Pork Steak Marinated in Fermented Dairy Products and Sous-Vide Cooked. *Sustainability* **2019**, *11*, doi:10.3390/su11205644.
29. Das, D.; Goyarl, A. Antioxidant activity and gamma-aminobutyric acid (GABA) producing ability of probiotic *Lactobacillus plantarum* DM5 isolated from Marcha of Sikkim. *LWT-Food Sci. Technol.* **2015**, *61*, 263-268, doi:10.1016/j.lwt.2014.11.013.
30. Bian, H.; Ma, J.; Geng, Z.; Liu, T.; Sun, C.; Wang, D.; Zhang, M.; Xu, W. Changes of hydroxyl-linoleic acids during Chinese-style sausage processing and their relationships with lipids oxidation. *Food Chemistry* **2019**, *296*, 63-68, doi:<https://doi.org/10.1016/j.foodchem.2019.05.183>.
31. Li, B.; Xu, Y.; Li, J.; Niu, S.; Wang, C.; Zhang, N.; Yang, M.; Zhou, K.; Chen, S.; He, L., et al. Effect of oxidized lipids stored under different temperatures on muscle protein oxidation in Sichuan-style sausages during ripening. *Meat Science* **2019**, *147*, 144-154, doi:<https://doi.org/10.1016/j.meatsci.2018.09.008>.
32. Sharedeh, D.; Gatellier, P.; Astruc, T.; Daudin, J.-D. Effects of pH and NaCl levels in a beef marinade on physicochemical states of lipids and proteins and on tissue microstructure. *Meat Science* **2015**, *110*, 24-31, doi:<https://doi.org/10.1016/j.meatsci.2015.07.004>.
33. Korzeniowska, M.; Kroliczewska, B.; Kopec, W. Carbonyl and sulfhydryl groups of chicken meat proteins after dietary modulation with selenium. *Open Chemistry* **2015**, *13*, 1293-1302, doi:10.1515/chem-2015-0147.
34. Zhang, L.; Lin, Y.H.; Leng, X.J.; Huang, M.; Zhou, G.H. Effect of sage (*Salvia officinalis*) on the oxidative stability of Chinese-style sausage during refrigerated storage. *Meat Science* **2013**, *95*, 145-150, doi:<https://doi.org/10.1016/j.meatsci.2013.05.005>.
35. Cheng, J.; Xu, L.; Xiang, R.; Liu, X.; Zhu, M. Effects of mulberry polyphenols on oxidation stability of sarcoplasmic and myofibrillar proteins in dried minced pork slices during processing and storage. *Meat Science* **2020**, *160*, 107973, doi:<https://doi.org/10.1016/j.meatsci.2019.107973>.
36. Zhao, C.J.; Schieber, A.; Gänzle, M.G. Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations – A review. *Food Research International* **2016**, *89*, 39-47, doi:<https://doi.org/10.1016/j.foodres.2016.08.042>.

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37. Sun, W.; Zhao, H.; Zhao, Q.; Zhao, M.; Yang, B.; Wu, N.; Qian, Y. Structural characteristics of peptides extracted from Cantonese sausage during drying and their antioxidant activities. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*, 558-563, doi:<https://doi.org/10.1016/j.ifset.2009.07.006>.
 38. Sante-Lhoutellier, V.; Aubry, L.; Gatellier, P. Effect of oxidation on in vitro digestibility of skeletal muscle myofibrillar proteins. *J. Agric. Food Chem.* **2007**, *55*, 5343-5348, doi:10.1021/jf070252k.
 39. Smid, E.J.; Kleerebezem, M. Production of Aroma Compounds in Lactic Fermentations. In *Annual Review of Food Science and Technology*, Vol 5, Doyle, M.P., Klaenhammer, T.R., Eds. Annual Reviews: Palo Alto, 2014; Vol. 5, pp. 313-326.
 40. Delgado, F.J.; González-Crespo, J.; Cava, R.; Ramírez, R. Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation. *International Dairy Journal* **2011**, *21*, 135-141, doi:<https://doi.org/10.1016/j.idairyj.2010.10.006>.
 41. Roldan, M.; Antequera, T.; Armenteros, M.; Ruiz, J. Effect of different temperature-time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. *Food Chemistry* **2014**, *149*, 129-136, doi:10.1016/j.foodchem.2013.10.079.