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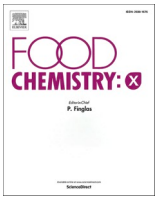

Effect of *Lactobacillus helveticus* IMAUJBH1 on fat and volatile flavor substances in fermented mutton sausages

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ABSTRACT

The decomposition and oxidation of fat is essential for the formation and quality of the unique flavor of sausage. To explore the effect of lactic acid bacteria on fat decomposition and oxidation in fermented sausage, free fatty acids and volatile flavor compounds were determined by gas chromatography (GC) and headspace solid-phase microextraction (HS-SPME)-GC–MS, respectively. The results showed that the addition of *Lactobacillus helveticus* IMAUJBH1 inhibited fat peroxidation and relatively increased the proportion of monounsaturated fatty acids. A total of 47 volatile flavor compounds were detected, including aldehydes, esters, alcohols, and ketones. The content of substances such as hexanal, heptanal, nonanal and 1-octene-3-ol related to lipid oxidation was significantly reduced. The results obtained in this study show that the strain can further affect the flavor of the product by inhibiting the formation of lipid oxidation or peroxide flavor substances to a certain extent.

1. Introduction

Fermented sausage is a traditional fermented meat product in China, that is highly favored by consumers due to its unique fermented flavor (Hu et al., 2022). However, due to the long production cycle of naturally fermented sausage and its richness in nutrients, it is susceptible to microbial contamination, which affects the quality characteristics of the fermented sausage itself. Lactic acid bacteria are the first microorganisms isolated from fermented meat products, and they are important advantageous flora in traditional fermented meat products, and are closely related to product quality (de Souza, de Oliveira, & de Oliveira, 2022).

Fat is one of the important components in fermented meat products and is mainly responsible for the desirable or undesirable flavors and aromas in meat products (Huang, Li, Huang, Li, & Sun, 2014; Wu et al., 2015). Hydrolysis and oxidation of fat in fermented sausages are two important biochemical reactions that are closely related to the flavor of fermented products, forming more than 80 % of the volatile flavor of the product (Ordóñez, Hierro, Bruna, & Hoz, 1999; Zhao et al., 2020). The catabolic process of fatty acid formation from triacylglycerols and phospholipids is an early step in the conversion of fats into volatile flavor compounds, while the oxidation of free fatty acids is the second step in their conversion to flavor substances (Jin et al., 2010). Numerous studies have pointed out that the lipase produced by lactic acid bacteria was beneficial to the increase of free fatty acid content in fermented sausages, especially by promoting the release of unsaturated fatty acids and providing basic reaction substances for the formation of flavor substances (Chen, Kong, Han, Xia, & Xu, 2017; Du et al., 2019; Xiao, Liu, Chen, Xie, & Li, 2020). On the one hand, unsaturated fatty acids undergo two oxidative pathways to produce hydroperoxides and hydroxy acids, because hydroperoxides are not stable, they can react further to form aldehydes, etc., and hydroxy acids can be oxidized further to form lactones with strong fruit flavors; on the other hand, saturated fatty acids are β-oxidized to produce ketoacyl coenzyme A, which in turn is enzymatically finalized to produce methyl ketones and secondary alcohols (Hu, Zhang, Wen, Chen, & Kong, 2022; Wang, Zhang, Liu, Jin, & Xia, 2022; Zhou et al., 2021).

Moderate levels of fat oxidation can have a positive impact on the formation of the typical flavor of meat products, but high levels of fat oxidation can generate reactive free radicals, which can negatively

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affect the color, texture, and nutritional value of the product, leading to off-flavors, rancidity that reduces the food value of the meat product, and even endangering human health (Falowo, Fayemi, & Muchenje, 2014; Marušić Radovčić, Poljanec, Petričević, Mora, & Medić, 2021).

However, due to the long production cycle of sausages, fat is more prone to peroxidation during the subsequent stages. Studies have pointed out that the primary oxidation products of fat oxidation are unstable, the secondary products of fat oxidation are more likely to interact with proteins. Malondialdehyde (MDA) is the most abundant active aldehyde in the secondary oxidation products of fat; it has a strong ability to induce oxidative deformation of proteins and is a marker of oxidative stress in fat (Domínguez et al., 2021; Zhou, Zhao, Su, Cui, & Sun, 2014). At the same time, some studies have pointed out that MDA has reaction characteristics with the free amino group of lysine, the guanidine group of arginine, the imidazole part of histidine, and the sulfhydryl group of cysteine to form various potentially toxic adducts (Vandemoortele & De Meulenaer, 2015).

With the continuous reports on fat oxidation and peroxidation in fermented meat products, researchers have begun to focus on reducing the degree of peroxidation to ensure the safety of products and the health of consumers. In addition, studies have shown that some lactic acid bacteria with antioxidant capacity can inhibit the autoxidation of fat in meat products and reduce the content of fat autoxidation products such as hexanal, octanal, and nonanal; they also inhibit excessive fat oxidation and reduce thiobarbituric acid reactive substances (TBARS) content (Wen, Kong, Yin, Zhang, & Chen, 2022; Zhang et al., 2017).

Therefore, controlling the fat oxidation of fermented sausages is a crucial aspect of their production and processing. Compared with the addition of artificial or natural antioxidants to reduce the degree of oxidation of sausages, the study of starter strains with antioxidant properties is a potential way to improve the quality of fermented sausages (Liu et al., 2023). In our previous studies, we screened *Lactobacillus helveticus* IMAUJBH1, which has good fermentation characteristics and antioxidant capacity, and used it as a fermenting agent in the production of fermented sausages. However, most of the previous studies focused on the basic indices of sausage and did not make a systematic study on fat oxidation and flavor of sausage. In this experiment, the free fatty acid content, lipoxygenase (LOX) activity, TBARS, and volatile flavor of fermented sausage were analyzed to investigate the effect of bacterial strains on fat oxidation in sausage based on previous studies. In addition, potential correlations between oxidation metrics and volatile flavors were explored to reveal the effect of fat oxidation on flavor. This study provides a theoretical basis for the development of new fermenters and directed product development.

2. Materials and methods

2.1. Materials and reagents

Sheep hind leg meat (the hind leg meat of 6–8 months old Sunit lambs) was purchased from Inner Mongolia Grassland Jingxin Food Company; sheep tail oil was purchased from Dongwayao Market in Hohhot, Inner Mongolia; collagen casings were purchased from Liuzhou Hongsheng Collagen Casing Company; sodium nitrite was purchased from Hangzhou Longshan Chemical Company; and glucose and soy protein isolates were purchased from Shandong Linyi Shansong Biological Products Company.

Dithiothreitol and linoleic acid were purchased from McLean Reagent Company, and the rest of the drugs were purchased from Sinopharm Chemical Reagent Company.

2.2. Strain resources

Lactobacillus helveticus IMAUJBH1 (strain number: CGMCC NO.24177) was obtained from the Microbiology Laboratory of Meat Science and Technology Team, College of Food Science and Engineering, Inner Mongolia Agricultural University, China.

2.3. Preparation of fermented mutton sausage

Two batches of mutton-fermented sausage were produced. One batch was inoculated with *Lactobacillus helveticus* IMAUJBH1 (10^9 CFU/g, inoculated 4 %) for fermentation as the experimental group (JBH1), and the other batch was not inoculated for natural fermentation as the control group (ZR).

According to the ratio of Sunite sheep hind leg meat: sheep tail oil = 7:3, 2.2 % salt, 0.5 % sucrose, 0.5 % glucose, 0.2 % white pepper, 0.05 % VC, 0.2 % dried ginger powder, 1 % white wine, 0.01 % sodium nitrite, 2 % soy protein isolate, and 4 % bacterial liquid were added. The production and processing were carried out according to the process flow of Fig. 1. Two batches of mutton fermented sausages were sampled and tested at 0 d (marination), 1 d (fermentation), 15 d (maturation), 30 d (storage for 15 d after maturation), and 45 d (storage for 30 d after maturation).

2.4. Determination of free fatty acids

2.4.1. Extraction of fatty acids

Fermented mutton sausage samples were treated according to the method of Folch, Lees, and Sloane Stanley (1957). Fermented sausages at different stages were taken, fully chopped after removing the casing, added to a mixture of $CHCl_3$-CH_3OH (V: V = 2:1), fully mixed and shaken for 2 h, and extracted for 8–10 h. Filtration was performed with a G3 funnel, and 5 mL 20 % NaCl solution was added to the filtrate, which was allowed to stand for stratification. Fat was extracted from the lower layer of fat extract and dehydrated by anhydrous Na_2SO_4, then concentrated by rotary evaporation at 40 °C, and fat saponification was performed by adding 5 mL of 0.5 mol/L NaOH-CH_3OH solution and refluxing at 70 °C for 5 min. Then, 5 mL of $BF_3{\cdot}C_2H_5OC_2H_5$ solution was added and refluxed at 70 °C for 2 min for fat methylation. Finally, add 2 mL of hexane (GC level), reflux at 70 °C for 1 min, add 5 mL of saturated NaCl solution, let stand for 10 min, aspirate 1 mL of hexane layer and filter with 0.22 μm organic filter membrane, place in the injection bottle, and store at −80 °C to be used.

2.4.2. Composition of fatty acids

Fatty acids were analyzed using a gas chromatograph (GC) (Agilent 8860, USA). The sample peak was determined by the retention time of the mixed standard of Sigma-Aldrich 37 fatty acid methyl esters, and the fatty acids were quantified (mg/kg meat) with methyl nonadecanoate as the internal standard.

GC conditions: Rt-2560 capillary chromatographic column (0.20 μm, 100 m × 0.25 mm), carrier gas (helium) flow rate of 1 mL/min, injection temperature of 240 °C, injection volume of 1 μL, split ratio of 100:1.

Temperature programming: initial temperature 100 °C, stable for 5 min; continue to heat up to 170 °C, the rate is 3 °C/min, stable for 10 min, continue to heat up to 220 °C, the rate is 3 °C/min; after 5 min of stabilization, the temperature was continuously raised to 240 °C at a rate of 1 °C/min, and the heating was completed after 10 min of stabilization.

2.5. Determination of thiobarbituric acid reactive substances

Referring to the method of Zhuang et al. (2022) with appropriate modifications. An accurately weighed 5.0 g of sausage meat sample was minced and added to 50 mL of a trichloroacetic acid mixture containing 0.1 % EDTA and shaken at 50 °C for 30 min in a constant temperature shaker. Remove and cool to room temperature, and filter twice through a double layer of filter paper. Take 5 mL of supernatant and add 0.02 MTBA solution, boiling in a water bath for 40 min, cooling, and centrifugation at 1600 r/min for 10 min. Add 5 mL$CHCl_3$ to the supernatant, let it stand and stratify, and measure the absorbance at 532 nm and 600 nm, respectively. The results were expressed as

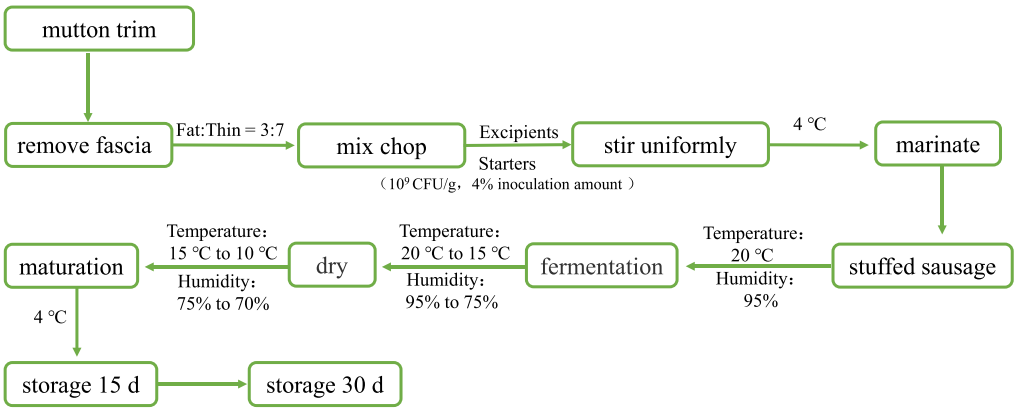

Fig. 1. Production process flow chart of fermented sausages.

malondialdehyde (MDA) mg/100 g sample. TBARS values were calculated according to the following formula.

TBARS values/(mg MDA/100 g) = (A532 − A600)*(1/5)*72.06*100

In the formula: 5 is the quality of the sample; 72.06 is the relative molecular mass of MDA; 155 is the molar absorption coefficient (L/(mol·cm)); 100 means 100 g sample.

2.6. Determination of lipoxygenase activity

2.6.1. Extraction of crude enzyme solution

Lipoxygenase activity in sausages was determined according to the method of Zhao et al. (2020) and Wang, Zhang, et al. (2022). Chopped sausage (2.0 g) were homogenized in 15 mL of phosphate buffer solution (50 mM, pH 7.0) containing dithiothreitol (1 mM) and ethylenediaminetetraacetic acid (1 mM), and centrifuged for 1 h at 4 °C and 10,000 g to collect the supernatant as the crude enzyme solution and set aside.

2.6.2. Enzyme activity determination

Following the method of Xu et al. (2018), 140 mg of linoleic acid was accurately weighed and dissolved in 5 mL of deoxygenated distilled water (containing 180 μL of Tween 20), adjusted to a pH of 9.0 with 2 M NaOH solution, diluted to 50 mL to make a substrate solution and stored at 4 °C under nitrogen conditions. The reaction system consisted of 2.8 mL citrate buffer (50 mM, pH = 5.5), 0.1 mL substrate solution, and 0.1 mL crude enzyme solution. The absorbance increment of 1 min was measured at 234 nm, an absorption increment of 0.01 units per minute was defined as one unit of LOX activity, and the activity associated with a sample was expressed as U/g sample.

2.7. Determination of volatile flavor

The volatile compounds were determined by headspace solid phase microextraction (HS-SPME) – ISQ single quadrupole gas chromatography (TRACE 1300) – mass spectrometry system (ISQ GC–MS system, Thermo, USA).

Referring to the method of Luo et al. (2019), a certain concentration of 2-methyl-3-heptanone solution was added to the sample, adsorbed at 60 °C for 40 min in a water bath, and then resolved and attached at 250 °C for 3 min in the gas chromatography inlet.

GC conditions: TR-5 capillary column (30 m × 0.25 mm, 0.25 μm); carrier gas He; carrier gas flow rate 1.0 mL/min; transmission line temperature 250 °C; unsplit stream sampling; injection time 1 min. Heating process: 40 °C (3 min), rate: 4 °C/min, the temperature was increased to 150 °C (1 min), then to 200 °C at 5 °C/min, and finally to 230 °C at 20 °C/min, holding the inlet temperature at 250 °C for 5 min.

MS conditions: the ion source temperature was 250 °C, the inlet temperature was 250 °C, the mass scanning range was *m/z* 30 to 400; and the solvent delay time was 1.0 min. The mass spectrometry data were searched and characterized with Meanlib, NISTDemo and Wiley Library, the identification was based on a match of >800. The results were expressed as μg/kg meat.

2.8. Statistical analysis

The data obtained from the experiment were processed using IBM SPSS Statistics, and one-way analysis of variance (ANOVA) was used to determine the significance of the differences, with $P < 0.05$ as the level of significance, and utilized for graphing with Origin 2021. Data are expressed as the mean ± standard deviation (SD) of three replicate determinations, and correlation parameters were analyzed by Pearson correlation analysis.

3. Results and discussion

3.1 Changes in fatty acid composition during processing

The fatty acid composition as well as the percentage of fatty acid content at different stages of fermented sausages are shown in Table 1 and Fig. 2, where a total of 13 saturated fatty acids (SFA), 8 monounsaturated fatty acids (MUFA) and 8 polyunsaturated fatty acids (PUFA) were detected and analyzed in combination.

The percentage of SFAs in all fatty acids showed a decreasing trend in the overall picture, and the proportion of the JBH1 group was lower than that of the ZR group in the first three stages of fermentation sausage, but significantly higher than that of the ZR group in the later stages. C16:0 is a fatty acid with high content in sausage. It showed a trend of decreasing first and then increasing, which was consistent with the change trend of SFA. Therefore, the change of SFA may be related to the change trend of C16: 0 content (Huimin et al., 2018).

This may be due to the fact that after the fermentation stage, the large-scale reproduction of lactic acid bacteria produces related enzyme substances to promote C16: 0 or SFA to produce coenzymes through the β-oxidation pathway, and finally produce alcohols and other substances, resulting in a decrease in its content (Wang, Zhang, et al., 2022). The increased of the content in the later stage may be due to the decrease of the activity of lactic acid bacteria, the inhibition of transformation, and the oxidation of MUFA or PUFA to SFA, resulting in a certain degree of increase in its content. At the end of 45 d of sausage storage, there were no significant changes in C4:0, C10:0, C12:0, C14:0, and C22:0 between the two groups, and the differences between the two groups were not significant. C15:0 and C23:0 were significantly lower in the JBH1 group than in the ZR group, and C23:0 was detected only at one stage in the ZR group and not in the JBH1 group; however, C6:0, C16:0, C17:0, C18:0, and C21:0 were significantly higher than those in the ZR group ($P <$

Table 1
Fatty acid composition of fermented sausages at different stages (mg/kg meat).

Fatty acids	Groups	Different stages (d)				
		0	1	15	30	45
SFA	ZR	2585.14^{Aa}	2391.44^{Aa}	930.05^{Bb}	740.54^{Bbc}	506.01^{Bc}
	JBH1	2203.85^{Aa}	2199.04^{Aa}	1171.64^{Ab}	1449.09^{Ab}	1411.79^{Ab}
C4:0	ZR	3.09 ± 0.10^{Ab}	3.19 ± 0.07 ^{Ab}	3.69 ± 0.58 ^{Ab}	4.54 ± 0.92 ^{Ab}	6.32 ± 0.82 ^{Ab}
	JBH1	3.19 ± 0.70 ^{Ab}	3.00 ± 0.48 ^{Ab}	3.80 ± 0.11 ^{Ab}	5.48 ± 0.04 ^{Ab}	7.75 ± 1.06 ^{Ab}
C6:0	ZR	537.71 ± 83.97^{Aa}	249.96 ± 104.32^{Ab}	115.90 ± 7.24^{Ac}	20.84 ± 1.11^{Bd}	135.35 ± 13.77^{Bc}
	JBH1	199.41 ± 28.45^{Bb}	8.52 ± 0.92^{Bc}	7.27 ± 0.11^{Bc}	151.03 ± 5.62^{Ab}	870.01 ± 91.00^{Aa}
C8:0	ZR	–	–	–	–	–
	JBH1	38.36 ± 22.25^{Aa}	–	–	–	–
C10:0	ZR	7.18 ± 0.31^{Ad}	7.00 ± 1.09 ^{Ad}	12.96 ± 4.37^{Ac}	10.52 ± 0.35^{Acd}	14.52 ± 4.49^{Abc}
	JBH1	6.45 ± 1.24 ^{Ad}	7.68 ± 1.01 ^{Ad}	17.54 ± 4.60^{Ab}	10.70 ± 1.08^{Acd}	13.93 ± 2.61^{Abc}
C12:0	ZR	5.69 ± 0.61^{Ac}	5.41 ± 0.87 ^{Ac}	9.93 ± 3.41^{Aab}	8.39 ± 0.46^{Bb}	11.26 ± 0.70^{Aa}
	JBH1	6.17 ± 1.51 ^{Ac}	6.49 ± 0.67 ^{Ac}	–	9.81 ± 0.82^{Abc}	12.31 ± 1.97^{Aab}
C13:0	ZR	15.47 ± 0.38^{Ab}	5.10 ± 0.38^{Bbc}	–	–	–
	JBH1	24.17 ± 17.04 ^{Ab}	45.78 ± 1.15^{Aa}	9.94 ± 4.50^{Acd}	–	–
C14:0	ZR	101.69 ± 8.09^{Ac}	96.85 ± 17.03^{Ac}	135.77 ± 18.45^{Bb}	140.64 ± 7.91^{Ab}	189.01 ± 9.88^{Aa}
	JBH1	101.22 ± 26.66^{Ade}	110.88 ± 11.52^{Ade}	298.74 ± 9.93^{Aa}	154.70 ± 11.61^{Acd}	212.76 ± 27.74^{Ab}
C15:0	ZR	44.15 ± 2.74^{Ac}	42.90 ± 6.38^{Ac}	64.58 ± 6.93^{Ba}	63.05 ± 1.85^{Aa}	47.92 ± 1.32^{Abc}
	JBH1	41.68 ± 10.17^{Ad}	45.25 ± 5.32^{Acd}	125.02 ± 3.40^{Aa}	63.08 ± 4.33^{Ab}	17.08 ± 1.37^{Be}
C16:0	ZR	925.82 ± 72.85^{Ab}	915.02 ± 130.15^{Ab}	21.52 ± 5.70^{Ad}	13.80 ± 0.93^{Bd}	26.79 ± 0.76^{Bd}
	JBH1	901.93 ± 212.49^{Aa}	980.75 ± 79.91^{Aa}	27.32 ± 8.86^{Ab}	956.28 ± 176.55^{Aa}	83.37 ± 6.05^{Ab}
C17:0	ZR	112.53 ± 9.09^{Ac}	110.02 ± 12.31 ^{Ac}	220.25 ± 5.36^{Ba}	88.19 ± 2.68^{Ad}	53.59 ± 6.42^{Be}
	JBH1	110.72 ± 19.82 ^{Ac}	108.13 ± 12.39 ^{Ac}	326.14 ± 9.50^{Aa}	49.48 ± 3.90^{Be}	81.12 ± 11.03^{Ad}
C18:0	ZR	569.54 ± 49.02^{Aa}	578.27 ± 69.50^{Aa}	19.07 ± 8.27^{Ad}	19.31 ± 1.71^{Ad}	–
	JBH1	511.28 ± 121.61^{Aa}	522.63 ± 2.01^{Aa}	17.10 ± 2.85^{Acd}	–	72.32 ± 4.85^{Acd}
C21:0	ZR	19.71 ± 1.76^{Aa}	15.14 ± 5.34^{Ba}	18.96 ± 4.68^{Aa}	19.17 ± 2.31^{Aa}	–
	JBH1	23.16 ± 3.83^{Aa}	45.93 ± 4.67^{Aa}	24.00 ± 5.37^{Ac}	–	21.25 ± 3.79^{Ac}
C22:0	ZR	242.56 ± 20.82^{Ac}	323.76 ± 43.92^{Aab}	307.43 ± 29.25^{Ab}	352.10 ± 15.64^{Aa}	21.26 ± 2.02^{Ad}
	JBH1	236.11 ± 5.18^{Ab}	313.99 ± 50.33^{Aa}	298.35 ± 17.40^{Aa}	48.55 ± 2.18^{Bc}	19.90 ± 1.66^{Acd}
C23:0	ZR	–	38.84 ± 4.21^{Aa}	–	–	–
	JBH1	–	–	–	–	–
MUFA	ZR	2134.63^{Ac}	2147.88^{Ac}	3654.05^{Ba}	2939.31^{Ab}	599.30^{Bd}
	JBH1	2228.16^{Ab}	2275.33^{Ab}	4836.25^{Aa}	1132.15^{Bd}	1667.22^{Ac}
C14:1	ZR	5.23±0.68^{Ade}	7.42±2.32^{Ad}	17.06±7.08^{Ac}	11.15±0.24^{Acd}	–
	JBH1	6.46±1.65^{Ae}	5.68±0.79^{Ae}	25.39±2.75^{Ac}	10.92±0.75^{Ade}	41.17±7.20^{Ab}
C15:1	ZR	78.62±24.69^{Ab}	99.19±3.09^{Aa}	114.32±3.05^{Aa}	18.06±2.87^{Bd}	71.81±3.17^{Ab}
	JBH1	85.94±8.51^{Ab}	89.78±24.15^{Ab}	111.34±12.31^{Aa}	22.81±0.73^{Ac}	24.52±1.02^{Bc}
C16:1	ZR	80.53±5.20^{Ad}	71.43±14.03^{Ad}	135.16±49.54^{Ad}	39.57±2.46^{Ad}	304.11±9.61^{Bc}
	JBH1	92.17±21.16^{Ade}	85.01±14.01^{Ade}	143.24±10.26^{Ad}	35.10±1.24^{Be}	484.87±47.90^{Ac}
C17:1	ZR	78.01±5.39^{Ac}	81.33±12.45 ^{Ac}	156.85±21.59^{Ba}	126.06±5.30^{Ab}	166.71±16.80^{Aa}
	JBH1	86.01±23.81^{Ac}	88.38±12.45^{Ac}	261.50±14.69^{Aa}	125.32±8.97^{Ac}	200.23±27.72^{Ab}
C18:1T9	ZR	1496.92±128.63^{Aa}	1488.07±218.29^{Aa}	893.98±33.25^{Bb}	683.37±21.82^{Ac}	–
	JBH1	1540.00±359.02^{Aa}	359.06±43.25^{Bd}	1202.19±44.73^{Ab}	629.95±11.17^{Bcd}	16.31±1.22^{Ae}
C18:1C9	ZR	342.79±31.94^{Ad}	340.81±63.57^{Bd}	2282.85±131.40^{Ba}	2030.28±27.79^{Aa}	–
	JBH1	366.22±102.51^{Af}	1588.40±96.25^{Ab}	3032.45±146.15^{Aa}	–	839.73±45.75^{Ac}
C20:1	ZR	26.73±2.35^{Aa}	29.95±3.64^{Aa}	39.27±4.06^{Aa}	30.82±2.39^{Ba}	56.66±1.37^{Aa}
	JBH1	24.97±4.41^{Ae}	29.40±1.82^{Ae}	60.15±1.47^{Ad}	308.04±9.57^{Ab}	46.57±2.85^{Bde}
C24:1	ZR	25.81±2.46^{Aa}	36.16±12.94^{Aa}	14.55±0.44^{Aa}	–	–
	JBH1	26.40±2.76^{Ab}	29.63±7.13^{Ab}	–	–	13.83±1.47^{Ac}
PUFA	ZR	856.73^{Ad}	1027.52^{Ac}	266.55^{Ae}	1279.43^{Bb}	4659.75^{Aa}
	JBH1	817.94^{Ac}	952.54^{Ac}	253.72^{Ad}	2866.37^{Ab}	3886.34^{Ba}
C18:2T9	ZR	32.65±3.25^{Ad}	37.43±2.76^{Ad}	99.36±14.38^{Ad}	41.09±3.12^{Bd}	3966.27±53.66^{Aa}
	JBH1	32.75±7.44^{Ae}	33.92±4.17^{Ae}	75.42±5.06^{Be}	2411.06±160.76^{Ad}	3314.65±248.76^{Bb}
C18:2C9	ZR	737.70±65.54^{Ac}	798.84±64.63^{Ab}	16.79±3.27^{Af}	1104.06±13.34^{Aa}	156.12±31.14^{Ae}
	JBH1	645.98±117.17^{Ab}	738.58±70.69^{Aa}	19.20±9.82^{Ae}	63.71±14.87^{Bde}	138.34±7.61^{Ad}
C18:3N6	ZR	14.68±1.81^{Acd}	59.64±36.10^{Aa}	49.72±10.18^{Aab}	33.47±0.28^{Bbc}	63.33±10.73^{Aa}
	JBH1	30.60±25.70^{Ad}	24.01±6.70^{Ad}	57.22±17.24^{Abc}	44.32±1.14^{Acd}	60.34±3.47^{Abc}
C18:3N	ZR	20.48±1.79^{Ab}	19.36±2.56^{Ab}	43.25±5.39^{Aa}	43.84±1.16^{Aa}	50.30±10.35^{Aa}
	JBH1	20.30±3.83^{Ad}	23.32±4.07^{Ad}	48.90±11.45^{Aab}	36.83±1.75^{Bc}	45.34±4.75^{Abc}
C20:2	ZR	17.80±1.58^{Ad}	19.97±0.75^{Acd}	21.82±1.73^{Abc}	22.15±0.36^{Abc}	23.85±2.07^{Ab}
	JBH1	17.28±1.67^{Ad}	21.23±2.63^{Acd}	21.47±2.61^{Acd}	18.46±5.50^{Ad}	28.68±3.88^{Ab}
C20:3	ZR	8.01±0.71^{Ad}	46.55±26.35^{Ac}	10.34±0.18^{Ad}	–	363.45±32.02^{Aa}
	JBH1	21.61±20.51^{Ac}	34.74±2.29^{Ac}	–	267.10±64.58^{Ab}	271.16±15.39^{Bb}
C20:4	ZR	25.41±6.16^{Ab}	36.16±12.94^{Ba}	–	–	–
	JBH1	32.72±2.12^{Ab}	58.67±10.27^{Aa}	–	–	–
C22:6	ZR	–	9.57±0.60^{Bd}	25.28±1.76^{Bc}	3.81±0.47^{Aa}	36.41±2.68^{Aa}
	JBH1	16.69±8.31^{Ad}	18.06±4.95^{Acd}	31.51±3.82^{Aab}	24.89±1.44^{Bbc}	27.83±1.89^{Bb}

Note: Different uppercase letters indicate significant differences between groups; different lowercase letters indicate significant differences between stages ($P < 0.05$), "-" indicates no detection.

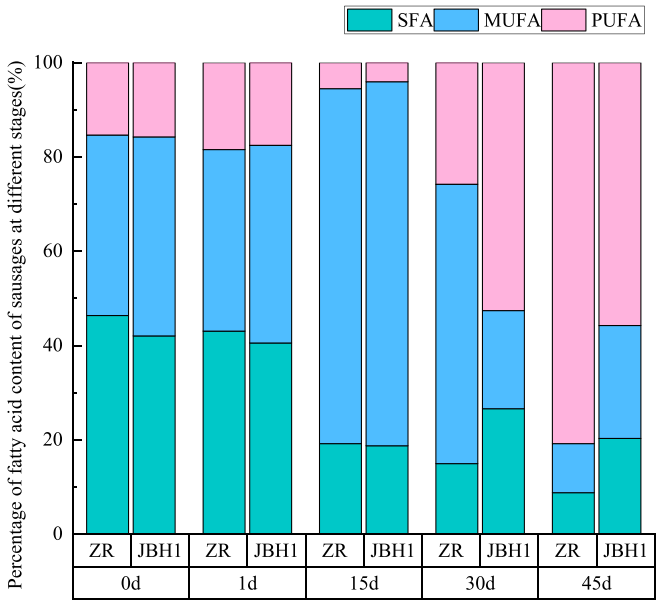

Fig. 2. Percentage of fatty acid content in different stages of fermented sausages.

0.05), and the latter two were detected only in the JBH1 group. It may be due to the fact that the added strains have certain lipase activity, which promoted the production of related fatty acids to a certain extent, resulting in the difference between the JBH1 group and the ZR group. For MUFAs, the proportion of the JBH1 group was higher than that of the ZR group at all but 30 d. The proportion of the two groups reached a maximum at 15 d, and the proportion of the JBH1 group was significantly higher than that of the ZR group at the end of the entire process. At the end of the entire sausage production process, C14:1, C16:1, C18:1, and C24:1 were significantly higher in the JBH1 group than in the ZR group, and it was hypothesized that the addition of the test strains could promote the production or accumulation of these MUFAs to a certain extent. At other stages, however, the two groups were essentially not significantly different, except for C18:1n9c. Studies have shown that the release of MUFA is derived from triglycerides. After bacterial fermentation, the degree of fat hydrolysis of the product can be improved. Unsaturated fatty acids were more easily released, and oleic acid and linoleic acid become the main fatty acids in sausages (Chen et al., 2017).

The PUFA proportions showed an overall trend of decreasing and then increasing, with little difference between the two groups in the first two phases, and the smallest proportions at 15 d. At the end of the fermented sausage production process, the PUFA percentage was significantly lower in the JBH1 group than in the ZR group. However, the proportion of MUFA was higher than that of ZR group, which may be due to the moderate oxidation of PUFA to produce a certain proportion of MUFA. At the end of 45 d, C20:4 was significantly higher in the JBH1 group than in the ZR group, whereas C18:2n6t, C20:3, and C22:6 were significantly lower than in the ZR group ($P < 0.05$), and there were no significant changes in C18:2n6c, C18:3n6, and C18:3n3. This may be because the content of C20: 3 and C22: 6 in the early stage was higher than that in the ZR group, and the oxidation reaction occurred in the 30-45d period to form part of SFA, resulting in a decrease in its content.

Overall, the experimental results proved that the addition of strain IMAUJBH1 could increase the proportion of MUFAs to a certain extent. That was to say, lactic acid bacteria fermentation could increase the lipolytic activity of bacteria, thereby increasing the release of fatty acids, which is in line with the findings of Chen et al. (2017).

3.2. Changes in TBARS value during processing

Generally, moderate fat oxidation produces volatile compounds that play an important role in flavor, but excessive fat oxidation can cause fermented meat products to lose good color and texture, produce rancid flavors, shorten the shelf life of the product, and even produce malondialdehyde, glutaraldehyde and other toxic substances (Marco, Navarro, & Flores, 2007; Mei, Pan, Guo, Ren, & Wang, 2022).

The TBARS value mainly indicates the generation of peroxidation products (malondialdehyde) from fat oxidation. As shown in Fig. 3, throughout the production and storage process, the changes in TBARS values of both groups showed a trend of increasing and then decreasing, and the TBARS values of the JBH1 group were always lower than those of the ZR group. This may be due to fermentation, rapid propagation of lactic acid bacteria, enhanced antioxidant activity, and the ability to produce antioxidant peptides in meat proteins, making the TBARS value of the JBH1 group lower than that of the ZR group (Jung et al., 2019). At 0 d, there was no significant change in the TBARS values of the two groups ($P > 0.05$), whereas at 1 d, the TBARS values of the JBH1 group were significantly lower than those of the ZR group ($P < 0.05$), and at 15 d, the TBARS values of the two groups had reached their maximum, which were 0.38 mg/100 g and 0.37 mg/100 g, respectively, and there was no significant difference between the two groups ($P > 0.05$). Then the TBARS value showed a downward trend.

The TBARS value mainly analyzes free carbonyl compounds, such as malondialdehyde (MDA), and its degradation mainly depends on factors such as temperature and time; at higher temperatures, it may undergo degradation, aldol condensation, or further react as a bifunctional group with proteins, peptides, amino acids, etc., in the sample to participate in nonenzymatic interactions to form nonenzymatic browning products (Sajib & Undeland, 2020). However, at lower temperature conditions, the reactivity of MDA itself will be reduced, and degradation may not be the first reaction pathway (Zhao et al., 2020).However, the fermented sausage is stored at 4 °C, so the possibility of MDA degradation caused by high temperature is small. It is speculated that MDA produced and accumulated during storage may be involved in nonenzymatic browning, resulting in a decrease in the TBARS value of sausages at 30 d and 45 d.

Compared with the JBH1 and ZR groups, the change trend of the two groups was basically the same. However, the TBARS value of the group with added strain was always lower than that of the ZR group, which proved that the addition of the strain played a certain role, which was consistent with the results of Mei et al. (2022).

Therefore, according to the above research conclusions, it is speculated that the decrease in the TBARS value at 30 d and 45 d may be due to the further reaction of MDA with other substances. However, during the whole production and storage process, the TBARS value of the JBH1 group was always lower than that of the ZR group, which proved that the addition of the strain would reduce lipid oxidation to a certain extent.

3.3. Changes in LOX activity during processing

LOX is an enzyme that requires Fe^{3+} to catalyze the generation of activity and can catalyze the generation of hydroperoxides from polyunsaturated fatty acids and lipids containing cis and cis 1,4-pentadiene structures, and further decompose them to form secondary oxidation products, which produce strong undesirable flavors and lead to the deterioration of meat products (Wang & Hammond, 2010).

As shown in Fig. 4, overall, LOX activity in the JBH1 group was consistently significantly lower than that in the ZR group ($P < 0.05$). At 0 d, the strain had not yet been fermented, and the LOX activity of the two groups of fermented sausage samples was not significantly different ($P > 0.05$). However, the LOX activity in the JBH1 group was significantly lower than that in the ZR group at 1 d after fermentation ($P < 0.05$). It was speculated that the strain rapidly propagated in the sausage to produce acid, which rapidly reduced the pH in the intestine and

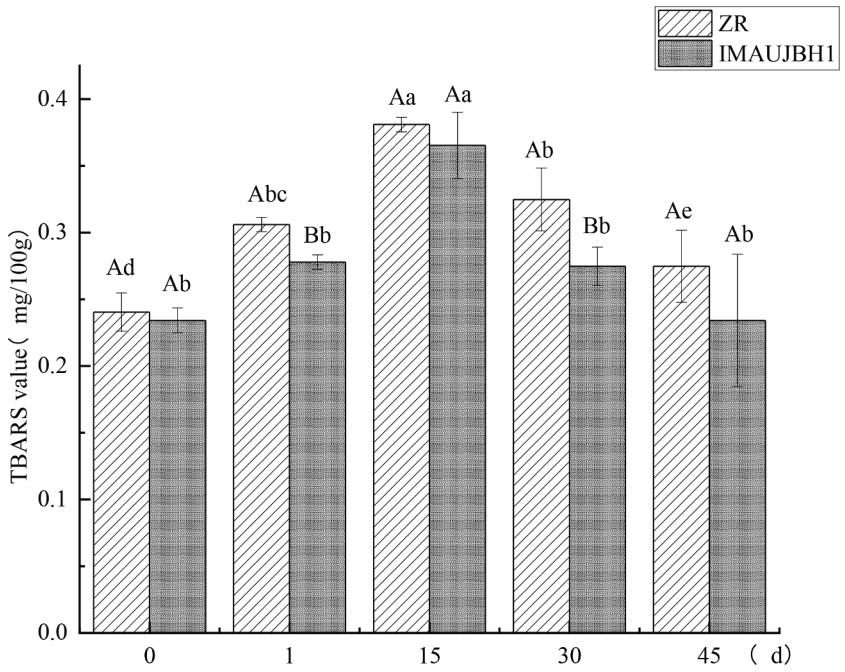

Fig. 3. Changes in TBARS values of sausages at different stages of the process. Note: Different uppercase letters indicate significant differences between groups; different lowercase letters indicate significant differences between stages ($P < 0.05$).

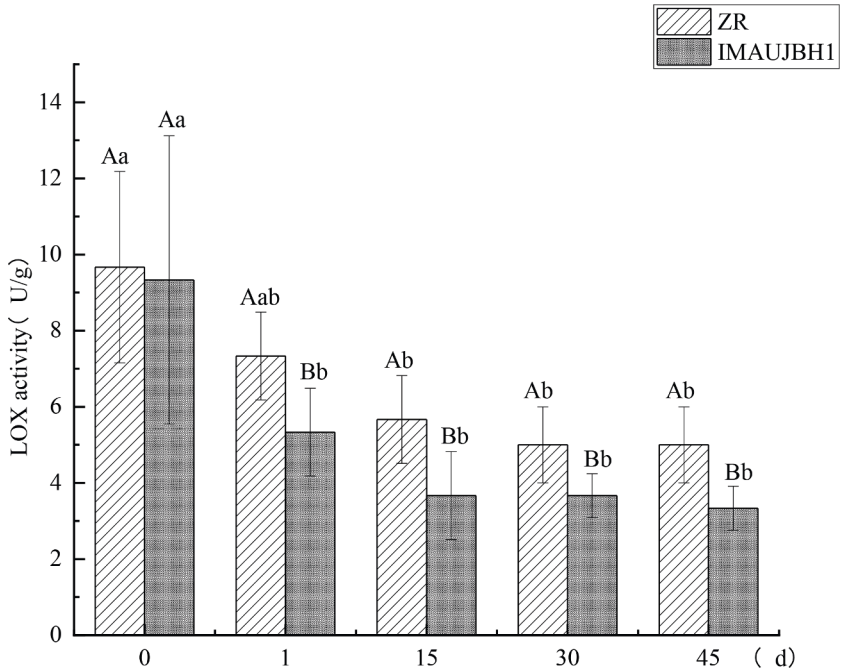

Fig. 4. Changes in lipoxygenase activity of sausage at different stages of the process. Note: Different uppercase letters indicate significant differences between groups; different lowercase letters indicate significant differences between stages ($P < 0.05$).

significantly inhibited LOX activity. At 15 d, the LOX activity of the two groups was still in a decreasing trend, and then the change tended to be stable, which may be due to the decrease in moisture after the sausage was dried and matured, which further affected the LOX activity. The results showed that inoculation of fermented sausages with *Lactobacillus helveticus* IMAUJBH1 could inhibit LOX activity to a certain extent and further inhibit fat oxidation. This is in agreement with Mei et al. (2022), who showed that *Lactiplantibacillus plantarum* with antioxidant properties can inhibit lipoxygenase activity and thus inhibit fat oxidation as well as the production of undesirable flavors.

3.4. Changes in volatile flavor compounds during processing

As shown in Table 2, a total of 47 volatile flavor compounds were detected during the production and storage of fermented sausages, including 9 aldehydes, 20 esters, 9 alcohols, 3 ketones, and 6 terpenes. Alcohols generally have a high odor threshold and have no direct effect on flavor, but they can indirectly affect the flavor of fermented sausages as reaction participants (Wang, Aziz, et al., 2022). As shown in Fig. 5 and Table 2, ethanol was the compound with the highest content in the early stage of fermented sausage, which may be related to the addition of

Table 2
The content of volatile flavor substances in fermented sausages at different stages (μg/kg meat).

Volatile compound	Groups	Different stages (d)				
		0	1	15	30	45
Aldehydes (9)						
Hexanal	ZR	0.21 ± 0.03^{Ad}	0.89 ± 0.19^{Aa}	0.48 ± 0.02^{Abc}	0.41 ± 0.24^{Abcd}	0.53 ± 0.08^{Abc}
	JBH1	–	0.24 ± 0.04^{Bbc}	0.39 ± 0.06^{Ba}	0.23 ± 0.05^{Abc}	0.22 ± 0.08^{Bc}
Heptanal	ZR	0.44 ± 0.08^{Aab}	0.32 ± 0.04^{Bc}	0.49 ± 0.12^{Aa}	0.28 ± 0.04^{Ac}	0.33 ± 0.07^{Abc}
	JBH1	0.24 ± 0.05^{Bb}	0.55 ± 0.07^{Aa}	0.22 ± 0.05^{Bb}	–	0.33 ± 0.05^{Ab}
Benzaldehyde	ZR	–	–	0.29 ± 0.05Aa	–	0.30 ± 0.04^{Ba}
	JBH1	0.19 ± 0.01^{Ac}	0.32 ± 0.04^{Aab}	0.36 ± 0.04^{Aab}	0.30 ± 0.09^{Aab}	0.37 ± 0.03^{Aa}
Phenylacetaldehyde	ZR	–	–	0.08 ± 0.03^{Aa}	0.07 ± 0.01^{Aa}	–
	JBH1	–	–	0.08 ± 0.02^{Aa}	–	–
(E)-2-Octenal	ZR	–	–	0.08 ± 0.02^{Aa}	–	–
	JBH1	–	0.13 ± 0.03^{Aa}	0.07 ± 0.01^{Ab}	0.09 ± 0.02^{Ab}	0.08 ± 0.01^{Ab}
Nonanal	ZR	2.71 ± 0.25^{Abcd}	2.21 ± 0.23^{Ad}	3.15 ± 0.09^{Aab}	2.13 ± 0.35^{Ad}	2.45 ± 0.24^{Acd}
	JBH1	2.57 ± 0.67^{Aa}	2.45 ± 0.09^{Aa}	2.06 ± 0.30^{Ba}	2.75 ± 0.75^{Aa}	2.53 ± 0.16^{Aa}
(E)-2- nonenal	ZR	0.27 ± 0.05^{Aab}	0.15 ± 0.02^{Bc}	0.33 ± 0.11^{Aa}	–	–
	JBH1	0.11 ± 0.03^{Bb}	0.34 ± 0.06^{Aa}	–	–	–
Decanal	ZR	0.25 ± 0.02^{Aa}	0.18 ± 0.02^{Ab}	0.15 ± 0.01^{Abc}	0.12 ± 0.01^{Ac}	0.16 ± 0.03^{Ab}
	JBH1	0.23 ± 0.0.3^{Aa}	–	–	0.11 ± 0.02^{Ab}	0.10 ± 0.01^{Bb}
Myristaldehyde	ZR	–	–	0.11 ± 0.01^{Bb}	0.19 ± 0.01^{Aa}	–
	JBH1	–	0.06 ± 0.01^{Af}	0.14 ± 0.05^{Ad}	0.20 ± 0.03^{Ac}	0.21 ± 0.01^{Abc}
Alcohols (9)						
Ethanol	ZR	4.8 ± 1.59^{Aa}	5.38 ± 2.54^{Aa}	6.89 ± 0.89^{Aa}	4.34 ± 2.49^{Aa}	3.88 ± 0.96^{Aa}
	JBH1	4.41 ± 2.43^{Aa}	4.62 ± 1.61^{Aa}	4.49 ± 1.88^{Aa}	5.23 ± 0.45^{Aa}	3.69 ± 0.62^{Aa}
Hexyl alcohol	ZR	–	–	–	–	
	JBH1	0.14 ± 0.04^{Ab}	0.28 ± 0.04^{Aa}	0.25 ± 0.03^{Aa}	–	–
3-Methyl-1-butanol	ZR	–	–	0.21 ± 0.04^{Aa}	0.17 ± 0.03^{Ab}	–
	JBH1	–	–	–	–	–
2-Heptanol	ZR	0.06±0.01^{Aa}	0.06±0.01^{Aa}	–	–	–
	JBH1	0.06±0.01^{Aa}	–	–	–	–
Heptanol	ZR	0.08±0.01^{Ad}	0.08±0.01^{Bd}	0.27±0.08^{Aa}	0.24±0.08^{Aab}	0.17±0.04^{Abc}
	JBH1	0.09±0.01^{Ae}	0.23±0.03^{Ab}	0.23±0.03^{Ab}	0.21±0.02^{Abc}	0.16±0.01^{Ad}
1-Octen-3-ol	ZR	0.47±0.11^{Ab}	0.46±0.12^{Ab}	0.46±0.04^{Ab}	0.45±0.02^{Ab}	0.41±0.06^{Ab}
	JBH1	0.33±0.15^{Aab}	0.45±0.01^{Aa}	0.37±0.03^{Bab}	0.30±0.10^{Bb}	0.33±0.01^{Bab}
1-Octanol	ZR	0.22±0.03^{Aab}	0.19±0.03^{Bbc}	0.27±0.04^{Aa}	0.21±0.06^{Abc}	–
	JBH1	0.22±0.03^{Abcd}	0.30±0.03^{Aa}	0.25±0.06^{Aab}	0.27±0.04^{Aab}	0.20±0.01^{Acd}
Linalool	ZR	0.43±0.25^{Aa}	0.59±0.22^{Aa}	0.24±0.01^{Bb}	0.22±0.01^{Ab}	0.20±0.02^{Bb}
	JBH1	0.53±0.05^{Aa}	0.29±0.05^{Bbc}	0.29±0.04^{Abc}	–	0.24±0.02^{Ac}
Citronellol	ZR	0.14±0.07^{Aa}	0.14±0.01^{Aa}	–	–	–
	JBH1	0.11±0.01^{Aa}	0.07±0.01^{Bb}	–	–	–
Esters (20)						
Ethyl acetate	ZR	0.13±0.04^{Ac}	2.26±0.18^{Ac}	0.47±0.06^{Abc}	–	0.49±0.02^{Bbc}
	JBH1	0.15±0.05^{Ad}	–	0.44±0.23^{Acd}	1.07±0.79^{Abc}	1.11±0.17^{Abc}
Ethyl butyrate	ZR	–	0.09±0.02^{Ba}	0.41±0.04^{Ab}	0.53±0.01^{Ab}	0.46±0.17^{Ab}
	JBH1	0.07±0.01^{Ad}	0.21±0.03^{Ac}	0.31±0.01^{Bbc}	0.27±0.13^{Bbc}	0.35±0.03^{Ab}
Ethyl lactate	ZR	–	–	1.39±0.14^{Abc}	1.58±0.06^{Ab}	1.52±0.25^{Bbc}
	JBH1	–	0.50±0.33^{Ac}	1.90±0.08^{Aa}	1.19±0.58^{Ab}	2.07±0.35^{Aa}
Ethyl isovalerate	ZR	–	–	0.24±0.05^{Abc}	0.23±0.02^{Abc}	0.23±0.03^{Abc}
	JBH1	–	–	0.10±0.01^{Bc}	–	0.11±0.01^{Bb}
Ethyl valerate	ZR	–	–	0.13±0.01^{Ab}	0.26±0.17^{Aa}	0.12±0.02^{Ab}
	JBH1	–	–	0.11±0.01^{Bb}	–	0.11±0.01^{Ab}
Ethyl caproate	ZR	2.46±0.07^{Ac}	2.26±0.18^{Bc}	4.46±0.28^{Ab}	4.51±0.19^{Ab}	3.96±0.48^{Ab}
	JBH1	1.92±0.19^{Bd}	3.56±0.14^{Ac}	3.99±0.28^{Bbc}	3.85±0.48^{Bbc}	4.15±0.21^{Ab}
Ethyl heptanoate	ZR	0.61±0.04^{Ade}	0.45±0.05^{Be}	1.36±0.29^{Abc}	1.58±0.57^{Acd}	1.15±0.13^{Acd}
	JBH1	0.38±0.19^{Bc}	0.90±0.27^{Abc}	1.25±0.33^{Aab}	1.06±0.15^{Ab}	1.43±0.58^{Aab}
Ethyl caprylate	ZR	1.88±0.25^{Ac}	1.74±0.28^{Bc}	3.84±0.60^{Aab}	4.11±0.24^{Aab}	4.55±0.69^{Aab}
	JBH1	1.52±0.23^{Af}	2.27±0.13^{Ae}	3.62±0.16^{Ac}	4.23±0.23^{Ab}	4.22±0.11^{Ab}
Ethyl benzoate	ZR	–	–	0.26±0.03^{Ab}	0.28±0.03^{Aab}	0.30±0.02^{Aa}
	JBH1	–	0.15±0.01^{Ae}	0.27±0.02^{Ac}	0.30±0.02^{Aab}	0.29±0.01 ^{Abc}
Ethyl phenylacetate	ZR	–	–	0.11±0.01^{Aa}	–	0.12±0.01^{Aa}
	JBH1	–	–	0.10±0.01^{Aa}	–	–
Ethyl nonanoate	ZR	0.79±0.13^{Ad}	–	1.04±0.25^{Abc}	1.08±0.09^{Ab}	1.25±0.19^{Aab}
	JBH1	0.72±0.07^{Ac}	0.76±0.07^{Ac}	0.86±0.07^{Ac}	1.19±0.16^{Aab}	1.06±0.05^{Ab}
Ethyl caprate	ZR	2.71±0.35^{Ad}	2.20±1.43^{Ad}	7.23±1.77^{Aab}	6.05±0.34^{Abc}	8.68±1.38^{Aab}
	JBH1	3.68±1.11^{Abc}	2.73±0.15^{Ac}	4.89±0.22^{Babc}	6.73±1.79^{Aabc}	9.96±6.21^{Aa}
Ethyl undecanoate	ZR	0.88±0.13^{Ab}	–	–	1.72±0.09^{Aa}	–
	JBH1	1.03±0.40^{Aab}	0.83±0.04^{Ab}	1.24±0.06^{Aa}	–	–
Ethyl laurate	ZR	0.12±0.01^{Ac}	0.16±0.02^{Ac}	0.33±0.08^{Aab}	0.37±0.02^{Aab}	0.48±0.11^{Aa}
	JBH1	0.17±0.05^{Ab}	0.15±0.01^{Ab}	0.27±0.02^{Ab}	0.51±0.16^{Ab}	0.39±0.01^{Ab}
Ethyl myristate	ZR	–	0.06±0.01^{Bd}	0.25±0.05^{Ab}	0.33±0.03^{Aa}	–
	JBH1	–	0.10±0.01^{Af}	0.21±0.02^{Ad}	0.37±0.03^{Ac}	0.35±0.01^{Ac}
Ethyl palmitate	ZR	0.17±0.09^{Ac}	–	0.23±0.04^{Abc}	0.25±0.08^{Abc}	0.50±0.18^{Aa}
	JBH1	0.23±0.14^{Ab}	0.30±0.02^{Ab}	0.28±0.02^{Ab}	0.25±0.08^{Ab}	0.27±0.05^{Bb}
Ethyl 2-ethylhexanoate	ZR	0.17±0.03^{Ab}	0.18±0.05^{Ab}	0.15±0.04^{Ab}	0.16±0.01^{Ab}	0.18±0.03^{Ab}
	JBH1	0.12±0.02^{Acd}	0.11±0.01^{Bd}	0.16±0.01^{Abcd}	0.21±0.07^{Aa}	0.18±0.01^{Aab}

(*continued on next page*)

Table 2 (*continued*)

Volatile compound	Groups	Different stages (d)				
		0	1	15	30	45
2-ethylhexyl acetate	ZR	–	–	–	0.25 ± 0.08^{Bc}	0.36 ± 0.06^{Ab}
	JBH1	–	–	0.27 ± 0.03^{Ad}	0.40 ± 0.07^{Abc}	0.37 ± 0.02^{Ac}
Ethyl 4-methyloctanoate	ZR	0.93 ± 0.20^{Ac}	0.96 ± 0.06^{Bc}	–	1.82 ± 0.04^{Bb}	2.30 ± 0.69^{Aab}
	JBH1	–	1.19 ± 0.12^{Ad}	–	2.06 ± 0.08^{Ab}	1.86 ± 0.15^{Ac}
Ethyl 5-methylnonate	ZR	–	0.12 ± 0.01^{Acd}	0.28 ± 0.09^{Abc}	0.35 ± 0.01^{Abc}	0.47 ± 0.29^{Aab}
	JBH1	–	0.11 ± 0.01^{Acd}	–	0.37 ± 0.24^{Ab}	0.31 ± 0.07^{Abc}
Ketones (3)						
2-Nonanone	ZR	0.16 ± 0.01^{Acd}	0.17 ± 0.04^{Abcd}	0.36 ± 0.05^{Aabc}	0.41 ± 0.12^{Aa}	–
	JBH1	0.11 ± 0.01^{Ba}	–	0.10 ± 0.01^{Bb}	–	–
3-Hydroxy-2-butanone	ZR	–	–	–	–	0.65 ± 0.34^{Aa}
	JBH1	–	–	0.36 ± 0.22^{Aa}	–	0.32 ± 0.12^{Aa}
2-Hendecanone	ZR	0.41 ± 0.09^{Aa}	0.36 ± 0.02^{Aa}	0.27 ± 0.02^{Ab}	0.25 ± 0.01^{Ab}	0.27 ± 0.03^{Ab}
	JBH1	0.33 ± 0.03^{Aa}	0.26 ± 0.02^{Bb}	0.23 ± 0.03^{Bbc}	–	0.20 ± 0.02^{Bc}
Terpenes (6)						
Camphene	ZR	0.16 ± 0.05^{Aa}	0.18 ± 0.03^{Aa}	0.15 ± 0.02^{Aa}	0.15 ± 0.05^{Ba}	0.13 ± 0.04^{Aa}
	JBH1	0.09 ± 0.01^{Bd}	0.07 ± 0.02^{Bd}	0.15 ± 0.05^{Abc}	0.18 ± 0.01^{Aa}	0.16 ± 0.01^{Aab}
Longifolene	ZR	0.42 ± 0.34^{Aab}	–	0.13 ± 0.04^{Ac}	–	0.48 ± 0.21^{Aa}
	JBH1	0.28 ± 0.10^{Aab}	0.28 ± 0.18^{Aab}	0.18 ± 0.03^{bc}	–	0.28 ± 0.03^{Aab}
1-caryophyllene	ZR	0.22 ± 0.11^{Aab}	–	0.10 ± 0.02^{Bbc}	0.11 ± 0.02^{Abc}	0.16 ± 0.05^{Abc}
	JBH1	0.60 ± 0.55^{Aa}	0.18 ± 0.06^{Aa}	0.20 ± 0.02^{Aa}	0.20 ± 0.14^{Aa}	0.16 ± 0.05^{Aa}
α-curcumene	ZR	2.30 ± 0.53^{Aa}	–	1.38 ± 0.09^{Abc}	1.33 ± 0.02^{Bc}	1.38 ± 0.19^{Abc}
	JBH1	2.18 ± 0.13^{Aa}	1.45 ± 0.11^{Ac}	–	1.59 ± 0.13^{Abc}	1.46 ± 0.15^{Ac}
β-sesquiphellandrene	ZR	1.71 ± 0.47^{Aa}	1.40 ± 0.23^{Aa}	0.92 ± 0.08^{Ab}	0.94 ± 0.05^{Ab}	0.90 ± 0.12^{Ab}
	JBH1	1.85 ± 0.33^{Aa}	1.18 ± 0.04^{Ab}	–	1.12 ± 0.15^{Ab}	1.10 ± 0.24^{Ab}
β-bisabolene	ZR	1.46 ± 0.38^{Aa}	–	0.72 ± 0.02^{Ab}	0.68 ± 0.01^{Ab}	0.69 ± 0.09^{Ab}
	JBH1	–	0.83 ± 0.07^{Aa}	–	0.92 ± 0.24^{Aa}	0.76 ± 0.10^{Aa}

Note: Different uppercase letters indicate significant differences between groups; different lowercase letters indicate significant differences between stages ($P < 0.05$), "-" indicates no detection.

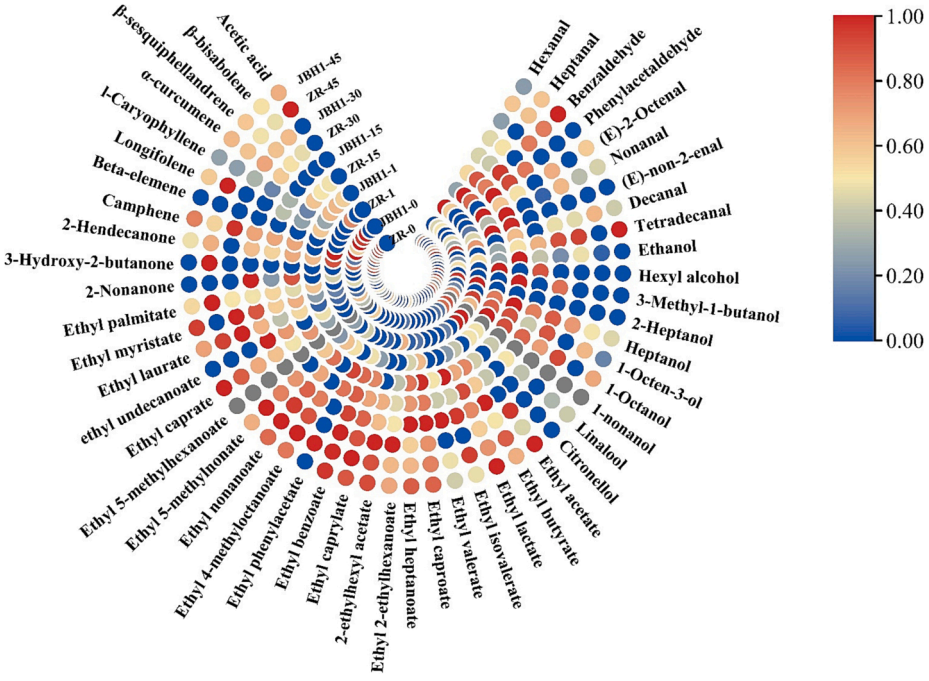

Fig. 5. Heat map of clustering of flavor substances in different stages of fermented sausages. Note: ZR-0 represents the sample of ZR Group 0 d, JBH1-0 represents the sample of JBH1 Group 0 d, ZR-1 represents the sample of ZR Group 1 d, JBH1-1 represents the sample of JBH1 Group 1 d, ZR-15 represents the sample of ZR Group 15 d, JBH1-15 represents the sample of JBH1 Group 15 d, ZR-30 represents the sample of ZR group stored for 15 d, JBH1-30 represents the sample of JBH1 group stored for 15, ZR-45 represents the sample of ZR group stored for 30 d, and JBH1-45 represents the sample of JBH1 group stored for 30 d.

liquor during the curing process of sausage. In addition, the added lactic acid bacteria can metabolize carbohydrates to produce substances such as ethanol through heterofermentation, resulting in higher ethanol content at 0 d and 1 d of fermented sausage. 1-Octen-3-ol is a product resulting from the oxidation of linoleic acid, which has a low odor threshold and can give fermented sausages a strong mushroom odor, in addition to the fact that its content reflects to some extent the degree of oxidation of sausage fats (Olivares, Navarro, & Flores, 2011). There was no significant difference in the content of 1-octen-3-ol between the two groups at 0 d and 1 d of fermentation of sausages ($P > 0.05$), but as the process continued to advance, the content of 1-octen-3-ol reached a maximum at 8 d in the two groups, which was 0.48 μg/kg in the ZR and

0.41 μg/kg in the JBH1. Subsequently, a decreasing trend was observed and the 1-octen-3-ol content in the JBH1 group was significantly lower than that in the ZR group ($P < 0.05$), which is speculated to be because the added strains inhibited the oxidation of the fats to a certain extent, as evidenced by the trend of the changes in the TBARS values above. This is also consistent with the findings of Chen et al. (2017).

Aldehydes are typical products of fat oxidation. Hexanal, heptanal and nonanal were the oxidation products of linoleic acid, n-6 and n-9 PUFA, respectively. They have a lower odor threshold, which can give the sausage grass flavor, fat flavor and orange flavor, and have a higher contribution to the flavor of fermented sausage. These compounds with moderate content can give the sausage a positive flavor (Cano-García, Rivera-Jiménez, Belloch, & Flores, 2014; Chen et al., 2017; Li, Cao, Yu, Zhu, & Zhao, 2023; Wen, Li, Han, Chen, & Kong, 2021; Xu et al., 2014). In the early stage of fermenting sausage, there was no significant difference between the contents of the three in the two groups ($P > 0.05$). With the processing stage, the contents of the three reached a maximum at the end of fermentation for 1 d or 15 d, and then showed a decreasing trend as a whole, and the content of the JBH1 group was significantly lower than that of the ZR group, which also reflected that the added *Lactobacillus helveticus* IMAUJBH1 could inhibit the autoxidation of fat to a certain extent.

Esters can be synthesized by esterification of alcohols with acids and by acetyltransferases (Dzialo, Park, Steensels, Lievens, & Verstrepen, 2017; Sun, Zhao, Zhao, Zhao, & Yang, 2010). Esters with a low odor threshold give fermented sausages a floral and fruity flavor, mask the putrid odor of the sausage, and play an important role in the flavor substances of fermented sausages (Hu et al., 2022). The content of ethyl lactate and ethyl acetate in the JBH1 group was significantly higher than that in the ZR group ($P < 0.05$), and the content of ethyl caproate increased rapidly and was significantly higher than that in the ZR group at the end of 1 d. Afterward, it showed a decreasing trend significantly lower than that in the ZR group ($P < 0.05$), and there was no significant difference between the two groups at 45 d ($P > 0.05$).

3.5. Correlation analysis of fat oxidation and volatile flavor

LOX is an enzyme that requires Fe^{3+} to catalyze the production of activity, which can increase the production of lipid hydroperoxides and promote the production of secondary oxidation products. The TBARS value reflects the amount of MDA in the secondary oxidation product. Both of them have a great influence on the wind characteristics of fermented sausage. Therefore we analyzed the correlation between the TBARS value, LOX activity, which is an indicator of fat oxidation, and volatile flavor of fermented sausages in this study. The results are shown in Fig. 6.

The results showed that the substances positively correlated with LOX mainly included: ethanol, ethyl undecanoate, 2-nonanone, 2-hendecanone, 1-octanol, heptanal, (E) −2-none, nonanal, 2-heptanol, 1-octen-3-ol, linalool, citronellol, and decanal; in particular, it was significantly positively correlated with 2-hendecanone ($P < 0.001$), citronellol, decanal, (E)-non-2-enal, 2-heptanol ($P < 0.01$), and 1-octen-3-ol ($P < 0.05$). However, there are no significant characteristics with ethanol, ethyl undecanoate, 2-nonanone, 1-octanol, heptanal, nonanal and linalool. It was found that LOX was positively correlated with the main aldehydes, alcohols and ketones, indicating that the related flavor substances would increase with the increase of LOX activity. If not properly controlled, it will lead to the emergence of negative flavor,

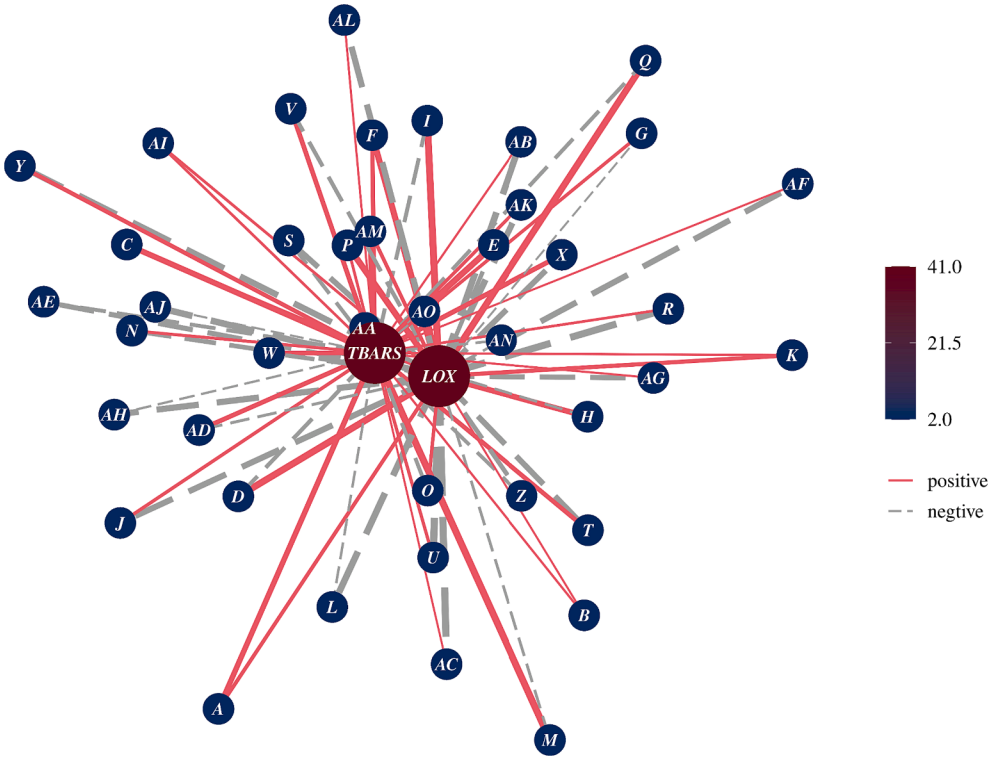

Fig. 6. Network diagram of correlation between oxidation indexes and volatile flavor substances in sausage. The red line in the figure indicates a positive correlation between the oxidation index and volatile flavor substances, while the gray line indicates a negative correlation, and the thickness of the line represents the strength of the correlation. Note: A = Ethanol; B = Hexyl alcohol; C = 3-Methyl-1-butanol; D = 2-Heptanol; E = Heptanol; F = 1-Octen-3-ol; G = 1-Octanol; H = Linalool; I = Citronellol; J = Hexanal; K = Heptanal; L = Benzaldehyde; M = Phenylacetaldehyde; N=(E)-2-Octenal; O = Nonanal; P=(E) −2-nonenal; Q = Decanal; R = Citronellol; S = Ethyl acetate; T = Ethyl butyrate; U = Ethyl lactate; V = Ethyl isovalerate; W = Ethyl valerate; X = Ethyl caproate; Y = Ethyl heptanoate; Z = Ethyl 2-ethylhexanoate; AA = 2-ethylhexyl acetate; AB = Ethyl caprylate; AC = Ethyl benzoate; AD = Ethyl phenylacetate; AE = Ethyl 4-methyloctanoate; AF = Ethyl nonanoate; AG = Ethyl 5-methylnonate; AH = Ethyl caprate; AI = Ethyl undecanoate; AJ = Ethyl laurate; AK = Ethyl myristate; AL = Ethyl palmitate; AM = 2-Nonanone; AN = 3-Hydroxy-2-butanone; AO = 2-Hendecanone.

which will have a negative impact on product quality. The increase in LOX activity leads to an increase in oxidation products, which is an important factor affecting the oxidation of sausage fats, which is in line with Mei et al. (2022), who suggested that "the increase in LOX activity increases lipid hydroperoxides, which decompose and form secondary oxidation products, resulting in a strong and undesirable flavor". And it is pointed out that lactic acid bacteria with antioxidant activity can maintain the inactive state of Fe^{2+} and inhibit the activity of lipoxygenase by cooperating with Fe^{2+}, thus inhibiting the production of bad flavor.

The substances that are positively correlated with TBARS mainly include: ethanol, ethyl undecanoate, 2-nonanone, 2-hendecanone, 1-octen-3-ol, 1-octanol, heptanal, (E)-2-nonenal, nonanal, ethyl caprylate, ethyl benzoate, ethyl phenylacetate, ethyl myristate, hexyl alcohol, 3-methyl-1-butanol, heptanol, hexanal, ethyl heptanoate, ethyl lactate, ethyl butyrate, (E)-2-octenal, ethyl valerate, ethyl caproate, phenylacetaldehyde, ethyl isovalerate, and ethyl 2-ethylhexanoate. Only phenylacetaldehyde ($P < 0.01$), heptanol, and 2-nonanone ($P < 0.05$) showed significant positive correlations, while the other substances showed positive but not significant correlations. Similarly, TBARS values were also positively correlated with the main aldehydes, alcohols, and ketones. The correlation results showed that the increase of its content would also affect the content of related flavor substances. However, the increase of TBARS value often indicates that fermented meat products lose good color and texture, resulting in rancid flavour (Marco et al., 2007; Mei et al., 2022). Therefore, controlling the TBARS value in fermented sausages is the basis for maintaining good product quality.

Therefore, the inoculation of functional lactic acid bacteria to control LOX activity and TBARS value is of great significance to improve the quality characteristics of fermented sausage.

4. Conclusion

The results of this study showed that the addition of lactic acid bacteria with antioxidant properties could affect the lipolytic oxidation of fermented sausages, resulting in some differences in their volatile flavor components. Pearson's algorithm showed that fat oxidation showed a positive correlation with flavor substances such as aldehydes and alcohols, which are essential for the influence of volatile flavor substances in products. Inoculation with IMAUJBH1 can reduce the content of aldehydes, alcohols and ketones, which are fat oxidation products, to a certain extent, and reduce the adverse effects of fat peroxidation on products. At the same time, IMAUJBH1 plays an important role in the sausage fermentation process, which not only accelerates the sausage production process, but also provides a certain guarantee for product safety. This work showed that the strain could reduce the accumulation of secondary products of fat oxidation in sausage to a certain extent and improve the flavor of the product, which could provide a theoretical basis for the directional development of the product.

CRediT authorship contribution statement

Fang Gao: Writing – original draft, Formal analysis, Data curation, Conceptualization. **Kaiping Zhang:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Daixun Wang:** Investigation, Formal analysis. **Lingyan Xia:** Investigation, Data curation. **Yue Gu:** Software, Investigation, Conceptualization. **Jianjun Tian:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Ye Jin:** Writing – review & editing, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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