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Dual-directional regulation of tea polyphenols on probiotic *Lactobacillus plantarum* and pathogenic *Staphylococcus aureus*, and its effect on quality of dry-fermented sausage

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Abstract

The growth of *Lactobacillus plantarum*, *Staphylococcus aureus*, and *Escherichia coli* in the presence of tea polyphenols (TP) was investigated using the methods of mono- and co-culture. The results showed that TP strongly inhibited *S. aureus* and *E. coli* even at a low concentration (0.2 mg/mL), but its effect on *L. plantarum* depended on the concentration used. TP displayed dual-directional regulation on the bacteria, which promoted the growth of prebiotic *L. plantarum* but simultaneously inhibited pathogenic *S. aureus* (and *E. coli*) at 2 mg/mL when they were co-cultured. This dual-directional regulation was further demonstrated by the production of dry-fermented sausages, and its effect on product quality was studied. These results indicated that 0.2% (w/w) TP could benefit *L. plantarum*, and suppress the growth of *S. aureus* in sausages. In addition, TP could inhibit the increase in TBARS and TVB-N values, and endowed products with a more attractive colour and enhanced flavour, while regulating the bacteria on sausages during fermentation. These results provided significant insights into the different effects of TP on bacteria, which would be beneficial for its application in sausages and similar fermented foods.

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Introduction

Fermented foods are a crucial pillar of societal traditions in the food industry, and probiotics play a significant role in their production process. Among the probiotics, *Lactobacillus* is the most commonly used (Grujovic *et al*., 2022), and can endow fermented foods with enhanced organoleptic and nutritional attributes (Macori and Cotter, 2018). Dry fermented sausage, a typical fermented food, is popular worldwide due to its unique taste. Its production is closely associated with *Lactobacillus*, which can improve flavour, and ameliorate quality through acid production, protein hydrolysis, and other multiple metabolic activities (Grujovic *et al*., 2022). Although many direct vat set starters of *Lactobacillus* are commercially available, dry fermented sausages are often prepared *via* natural folk fermentation pathways. In this traditional process, various undesired spoilage microorganisms, such as *Staphylococcus aureus* and *Escherichia coli*, usually appear with the probiotic *Lactobacillus*, consequently resulting in product quality loss and safety risks. Studies have shown that *S. aureus* and *E. coli* are responsible for food deterioration and many foodborne diseases that pose a significant threat to the food industry and public health (Gutierrez *et al*., 2016; Macori and Cotter, 2018). Therefore, the search for natural products that can simultaneously inhibit spoilage microorganisms, and promote the proliferation of probiotics is important for the natural fermentation of sausages.

Due to their antioxidant effects, tea polyphenols (TP) have been extensively used as additives in many areas of the food industry (Gramza and Korczak, 2005), including various fermented meat products such as Chinese sausage, ham, and cured meat (Wang *et al*., 2015; Ma *et al*., 2021). TP has also been shown to effectively inhibit several common foodborne pathogens, such as *S. aureus* and *E. coli* (Malongane *et al*., 2017). TP may exhibit broad-spectrum antibacterial activity at high concentrations, even against the probiotic *Lactobacillus*. Therefore, the concentration of TP

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used in food processing is crucial to inhibit spoilage microorganisms and promote *Lactobacillus* simultaneously. However, such concentration-dependent and dual-directional regulation on microorganisms, and the subsequent effects on the quality of fermented sausages, have rarely been researched.

In the present work, the effect of TP on the growth of *S. aureus*, *E. coli*, and *Lactobacillus plantarum* was investigated, and a TP concentration threshold was proposed to achieve dual-directional regulation on microorganisms in co-culture. This regulation was further demonstrated during the production of dry fermented sausages, accompanied by an improvement in flavour and product quality, including freshness, colour, and acidity. These results may promote the application of TP in sausages and similar fermented foods in consideration of product quality and safety.

Materials and methods

Strains and chemicals

Staphylococcus aureus ATCC 29213, *Escherichia coli* ATCC 43889, and *Lactobacillus plantarum* CICC 6253 were obtained from the China Centre of Industrial Culture Collection (Beijing, China). Tea polyphenols (TP, 98%) were sourced from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China), and their main components were epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate. Nutrient broth (NB), de Man-Rogosa-Sharpe broth (MRS), Baird-Parker agar (BP), nutrient agar (NA), and *Lactobacillus* Selective agar (LBS) were purchased from Chengdu Chron Chemicals Co., Ltd. (Chengdu, China).

Growth of strains with TP

Strains were activated three times at 37°C, and diluted with sterile phosphate buffer solution (PBS, pH 6.8) to approximately 5×10^7 CFU/mL before used. The diluted suspension (1 mL) was added to 50 mL of medium (MRS for *L. plantarum*, NB for *E. coli* and *S. aureus*) containing TP at concentrations of 0, 0.2, 0.8, 1, 2, 4, and 6 mg/mL. The mixtures were then incubated at 37°C and 150 rpm (28 h for *L. plantarum*, 12 h for *E. coli* and *S. aureus*). After incubation, the pH of the *L. plantarum* medium was determined. All media were diluted and plated. The plates were incubated at 37°C for 48 h for *L. plantarum* and 24 h for *E. coli* and *S. aureus*, and the viable count was expressed as log CFU/mL.

For the co-culture experiments, the suspensions of *L. plantarum* (1 mL) and *E. coli* (1 mL) were added to 50 mL of MRS broth in the presence of TP at concentrations of 0, 2, and 4 mg/mL, and incubated at 37°C and 150 rpm. The same procedure was performed for the co-culture of *L. plantarum* and *S. aureus*. Samples at incubation times of 0, 4, 8, 12, 16, 22, 28, and 34 h were collected to determine the pH, and then diluted and plated on LBS or NA for cultivation and colony counting, as earlier described.

Preparation of dry fermented sausage

Considering that *S. aureus* is more sensitive to TP than *E. coli*, *S. aureus* and *L. plantarum* were selected for inoculation into the sausages. Dry fermented sausages were prepared following the method reported by Chen *et al*. (2015) with slight modifications. The sausages were divided into the following four batches: Groups L and S were inoculated with 10^7 CFU/g *L. plantarum* and *S. aureus*, respectively. Groups L-TP and S-TP were the same as L and S, but with 0.2% (w/w) of TP added, respectively. The sausage formulation included 800 g of lean pork, 200 g of fat, 25 g of NaCl, 50 g of dextrose, 0.10 g of sodium ascorbate, 3.0 g of monosodium glutamate, and 3.0 g of mixed spices. Minced lean meat, fat, and other ingredients were mixed and filled into pig intestinal casings, and then ligated into 8 - 10 cm sections. Each batch of sausage (15 sections) was air-dried at room temperature for 1 d, and then transferred for fermentation (25°C, 80% relative humidity). At 0, 1, 3, 6, and 9 d, three sections of sausage from each batch were analysed.

Determination of Lactobacillus and S. aureus populations on sausage

The growth of *Lactobacillus* and *S. aureus* in the sausages was analysed. The three sections of the sausage samples were minced and fully mixed, and 5 g of the sample was suspended in 45 mL of sterilised saline solution (0.9% NaCl), followed by homogenisation (6,000 rpm, 30 s). The homogenate was used for colony counting as earlier described. *Lactobacillus* and *S. aureus* were cultivated on LBS and BP agar, respectively, and their counts were expressed as log CFU/g.

Analysis of sausage properties

Each section of sampled sausage was used for analysis, and the result was the average value of the three sections. TBARS values were determined following the methods described by Ying *et al*. (2016).

For TVB-N analysis, 5 g of minced sausage was added to 25 mL of distilled water, and homogenised at 10,000 rpm for 30 s. The homogenate was incubated at 120 rpm for 30 min, centrifuged, and the supernatant was analysed using the Conway micro-diffusion method. The value was expressed as mg of nitrogen in 100 g sausage (Li *et al*., 2021).

Water activity (a_w) was measured using a water activity meter (Labmaster aw STANDARD, Novasina, Switzerland), and pH was evaluated according to Zhang *et al*. (2017). The surface colour of the sausages was measured using a colorimeter (CM-5; Konica Minolta, Tokyo, Japan). For each sausage section, three points were randomly selected for measurement, and the parameters *L** (lightness), *a** (redness), and *b** (yellowness) were recorded.

Texture analysis was performed using a texture analyser (TA-XT plus; Stable Micro Systems, London, UK) fitted with a P/36R probe. At the centre of each sausage section with a thickness of 1 cm, it was warp-wise compressed to 50% at a crosshead speed of 1 mm/s, and the hardness, springiness, cohesiveness, and chewiness parameters were calculated.

Analysis of volatile compounds of sausage

The volatile compounds of the samples were determined using headspace-solid phase microextraction-gas chromatography-mass spectrometer (HS-SPME-GC-MS) (Lu *et al*., 2019). Briefly, 5 g of minced sausage sample was transferred into a headspace vial, and 10 µL of ethyl hexanoate solution (10 mg/L in methanal) was added as internal standard. With the incubation in a water bath at 60°C for 10 min, the minced sample was exposed to an SPME fibre (50/30 µm DVB/CAR/PDMS, Supelco, Inc., Bellefonte, PA, USA) for 30 min. Then, desorption of the SPME fibre was performed in a GC-MS injector at 250°C for 5 min. The GC-MS (QP2010 SE, Shimadzu, Co., Kyoto, Japan) was equipped with DB-5MS capillary column (30 m × 0.25 mm × 0.25 µm; J&W Scientific, Folsom, CA, USA), and the temperature program was as follows: 40°C for 3 min, 5°C/min to 130°C, 8°C/min to 200°C, 10°C/min to 260°C, and 260°C for 7 min. The temperature of the ion source was 230°C, helium (1.0 mL/min) was used as the carrier gas, and the ionisation energy, detector voltage, scan range, and scan rate were 70 eV, 350 V, 35 - 450 m/z, and 0.2 s/scans, respectively. The content of the volatile compositions was expressed as the relative concentration to internal standard.

Statistical analysis

All experiments were performed in triplicate. Data were presented as mean ± standard deviation, and analysed using SPSS Statistics 26 (SPSS Inc., Chicago, IL, USA) for One-way analysis of variance (ANOVA). Significant differences were determined using Duncan's test ($p < 0.05$). Figures were constructed using the Origin 2021 software (OriginLab Inc., Northampton, USA).

Results and discussion

Effect of TP on growth of L. plantarum, E. coli, and S. aureus

The effect of TP on *L. plantarum* was concentration-dependent (Figure 1A). When the concentration was less than 2 mg/mL, TP did not exert an obvious stimulatory effect on *L. plantarum* growth, whereas a significant increase in viable counts was observed with 2 and 4 mg/mL of TP (by 21.49 and 19.13%, respectively), indicating a clear prebiotic effect on *L. plantarum*. However, the growth of *L. plantarum* was remarkably suppressed when the concentration of TP was further increased to 6 mg/mL. Contrary to the colony count trend, the pH values of the cultures first decreased, and then increased with increasing TP concentration, ranging from pH 3.43 to 3.73 (Figure 1A). The presence of TP changed the pH of the cultures by affecting *L. plantarum*, with the highest proliferation at 2 mg/mL of TP, leading to the lowest pH. This might have been due to the fact that *L. plantarum* can accumulate many organic acids, such as lactic and acetic acids, to reduce the pH value (Zhang *et al*., 2013). The results showed that at an appropriate concentration, TP could have a stimulatory effect on the growth of *L. plantarum*, whereas a high concentration of TP could lead to an inhibitory effect. Similar studies found that some polyphenols had concentration-dependent effects on *Lactobacillus*. For example, *L. hilgardii* 5w could grow normally over a wide concentration range

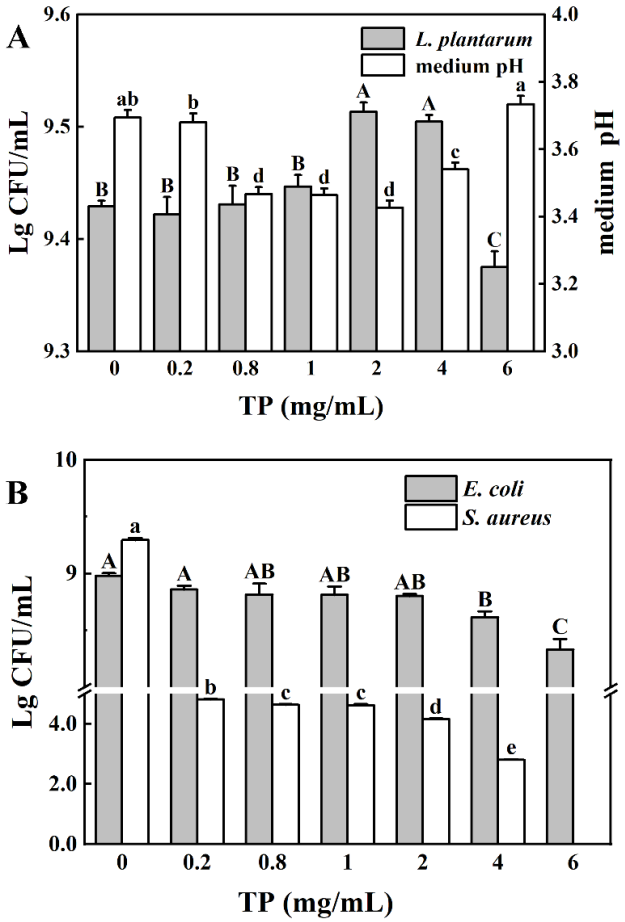

Figure 1. Effect of TP on growth of *L. plantarum* **(A)**, and *E. coli* and *S. aureus* **(B)** (10^6 CFU/mL, 37°C, and 28 h for *L. plantarum*; and 12 h for *E. coli* and *S. aureus*). Different letters indicate significant difference ($p < 0.05$).

of gallic acid (Alberto *et al*., 2001). An appropriate concentration of green tea extract promoted the viability of *Lactobacillus* (Shah *et al*., 2010). The growth-promoting effect of polyphenols on lactic acid bacteria (LAB) is complicated, and partly explained by their enhancing effects on metabolic functions and antioxidant ability to prevent membrane lipid peroxidation and protect membrane fluidity (Andrade *et al*., 2021).

Unlike *L. plantarum*, both *E. coli* and *S. aureus* showed a decreasing trend in viable counts with increasing TP concentrations (Figure 1B). In other words, TP inhibited the growth of *E. coli* and *S. aureus* even at a low concentration of 0.2 mg/mL. *S. aureus* was much more susceptible to TP than *E. coli*, which was consistent with the results of previous studies (Nakayama *et al*., 2011; Singh *et al*., 2016). *E. coli* displayed an obvious decrease in cell count when treated with 4 mg/mL TP, whereas *S. aureus* was considerably suppressed by 0.2 mg/mL TP. This might be because, unlike *E. coli*, *S. aureus* has no outer membrane covering the cell wall. Consequently, TP could damage the integrity of the cell wall of *S. aureus* more easily, thus hindering its metabolism and biosynthesis (Yoda *et al*., 2004; Shi *et al*., 2021).

Dual-directional regulation of TP during co-culture of L. plantarum with S. aureus (or E. coli)

The co-culture of *L. plantarum* with *S. aureus* (or *E. coli*) was conducted in the presence of TP to investigate its dual-directional regulation of microorganisms, namely, promoting the growth of *L. plantarum* and simultaneously inhibiting *S. aureus* and *E. coli*. When *L. plantarum* was co-cultured with *S. aureus* at an initial ratio of 1:1, *L. plantarum* showed a significant increase in count over time (Figure 2A), coupled with a general decrease in *S. aureus* cells (Figure 2B) and medium pH (Figure 2C),

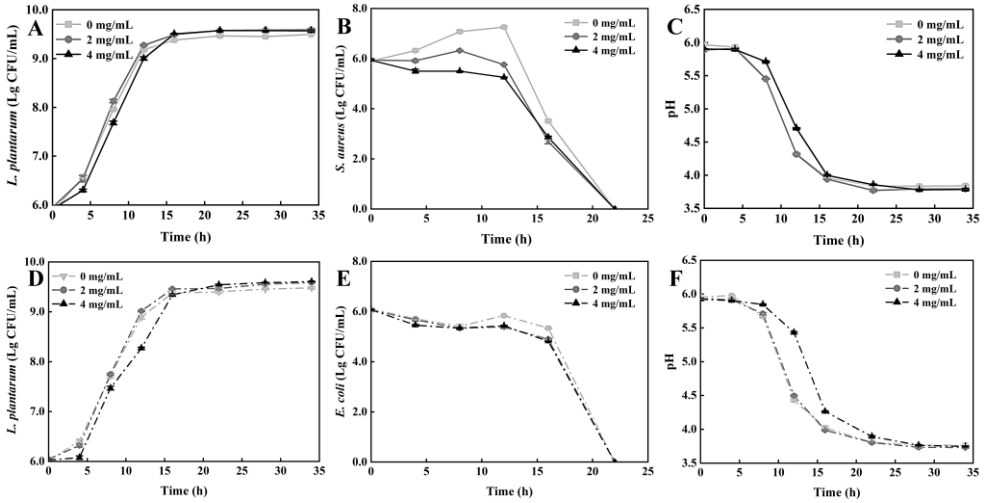

Figure 2. Dual-directional regulation effect of TP during co-culture of *L. plantarum* with *S. aureus* **(A - C)**, and *L. plantarum* with *E. coli* **(D - F)** (MRS, 37°C, and inoculation ratio 1:1).

regardless of the presence of TP. In the presence of 2 and 4 mg/mL TP, the cells of *L. plantarum* at the stationary phase were higher than in the absence of TP (Figure 2A), which was consistent with the results shown in Figure 1A. However, cultures with TP had fewer *S. aureus* cells than the control, and the cell counts displayed a sharp decrease after 12 h of co-cultivation with *L. plantarum* (Figure 2B). The pH of the medium varied mainly with the growth of *L. plantarum* during the co-culture, gradually decreasing over 15 h, and then stabilising over time (Figure 2C).

The results for *L. plantarum-E. coli* co-culture are shown in Figures 2D - 2F, which were very similar to those observed when *L. plantarum* was co-cultured with *S. aureus.* These observations indicated that at concentrations of 2 and 4 mg/mL, TP exerted dual-directional regulation on the prebiotic *L. plantarum*, as well as the pathogenic *S. aureus* and *E. coli* during co-culture. TP promoted the growth of *L. plantarum* while inhibiting the growth of both *S. aureus* and *E. coli*. This dual-directional regulation was also observed when the inoculation ratio of *L. plantarum* to *S. aureus* (or *E. coli*) was adjusted to 1:2. This suggested that TP supported the growth of *L. plantarum*, even when the pathogenic bacteria were initially dominant in the co-culture. Similarly, significant inhibition of *E. coli*, *Listeria monocytogenes*, and *Salmonella*, along with the simultaneous promotion of *L. rhamnosus*, was observed when these bacteria were co-cultured in raspberry-containing cultures enriched with polyphenols (Bauza-Kaszewska *et al*., 2021). The different susceptibilities of bacteria to polyphenols may be attributed to the fact that LAB are less sensitive to polyphenols, as they can synthesise galloyl-esterase and decarboxylase, metabolising some phenolic compounds to stimulate growth (Tabasco *et al*., 2011). However, polyphenols may damage the cell walls and DNA of many pathogenic bacteria, thereby inhibiting their growth (Bouarab Chibane *et al*., 2019). Moreover, LAB metabolites, such as bacteriocins and organic acids, could limit the multiplication of pathogenic bacteria during co-culture (Piekarska-Radzik and Klewicka, 2020).

Effect of TP on quality of sausage by dual-directional regulation on L. plantarum and S. aureus

Growth of Lactobacillus and S. aureus on sausage with TP

The dual-directional regulation of TP on bacteria, as earlier discussed, was further investigated in practical sausage production to study the effect of this action on the quality and safety of the product. In this experiment, prebiotic *L. plantarum* and pathogenic *S. aureus* were introduced into the production of dry fermented sausages in the presence of TP. During fermentation, the counts of naturally existing *Lactobacillus* and *S. aureus* in the sausages increased from 2.8 and 2.9 log CFU/g to 3.63 and 4.20 log CFU/g, respectively. The counts of *Lactobacillus* on sausages during fermentation are shown in Figure 3A. With the inoculation of 10^7 CFU/g of *L. plantarum*, the sausages maintained a high level of *Lactobacillus* during the 9-d

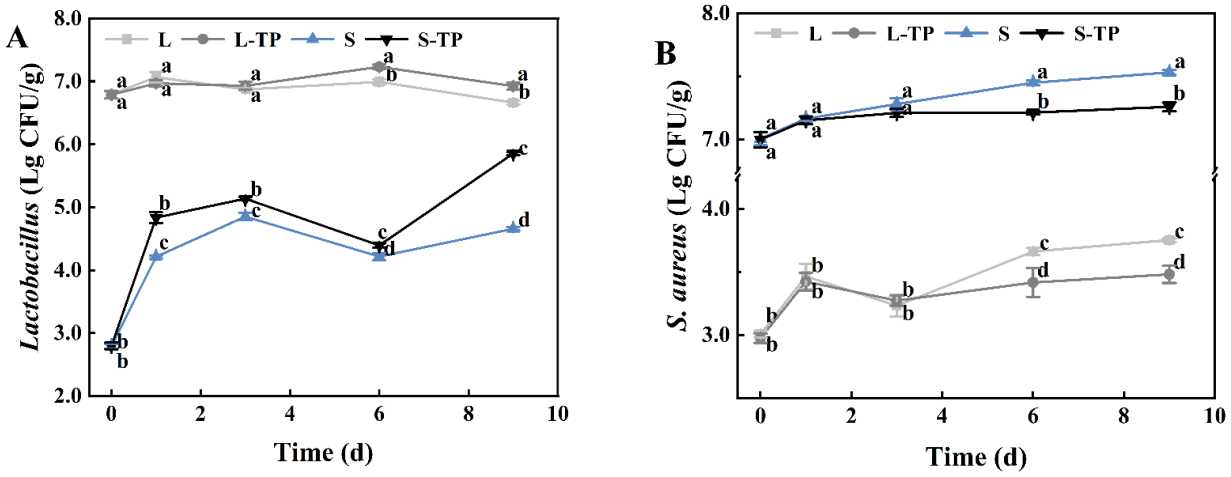

Figure 3. Growth of *Lactobacillus* and *S. aureus* on dry fermented sausage, with or without TP (L: *L. plantarum* inoculation 10^7 CFU/g; L-TP: L with 0.2% TP; S: *S. aureus* inoculation 10^7 CFU/g; and S-TP: S with 0.2% TP). Different letters on similar day indicate significant difference ($p < 0.05$).

fermentation (Figure 3A, L and L-TP), and the counts of *Lactobacillus* on sausages without *L. plantarum* (S and S-TP) increased from 2.79 log CFU/g to 4.66 and 5.85 log CFU/g, respectively. After fermentation, the number of *Lactobacillus* in the L-TP and S-TP groups was significantly higher than that in groups L and S, respectively, indicating that TP promoted the proliferation of *Lactobacillus* in dry fermented sausage. Xiang *et al*. (2019) reported that sausages incubated with mulberry polyphenols contained the highest number of *Lactobacillus* species during storage. As shown in Figure 3B, the counts of *S. aureus* in the L-TP and S-TP groups were significantly lower than those in the L and S groups during fermentation, indicating that TP suppressed *S. aureus.* These observations were consistent with the results of co-culture, as earlier discussed, demonstrating that TP could exert dual-directional regulation on *Lactobacillus* and *S. aureus* during the production of dry fermented sausage.

Physiochemical properties of dry fermented sausages

Excessive oxidation, which may form hazardous compounds, and lead to potential food safety risks, is one of the key factors responsible for the deterioration of meat product quality (Xu *et al*., 2021). TP exhibits the capacity to attenuate lipid oxidation in meat and maintain food quality. Based on our results (Figure 4A), the TBARS values of all the samples increased significantly during fermentation. On day 9, the group L-TP and S-TP showed lower TBARS values (0.49 and 0.44 mg MDA/kg, respectively), while sausage without TP yielded higher values of TBARS (0.57 and 0.65 mg MDA/kg, respectively), which indicated that lipid oxidation in dry fermented sausage was inhibited by TP. Alirezalu *et al*. (2017) showed that 500 ppm of green tea extract decreased lipid oxidation, and improved the quality of frankfurter sausages.

TVB-N is commonly considered a biomarker of protein and amine degradation, reflecting the freshness and shelf-life of meat products. As shown in Figure 4B, owing to bacterial spoilage, enzymatic spoilage, and oxidation, the TVB-N content in all sausages gradually increased during fermentation (Bekhit *et al*., 2021). Among all groups, the sausages inoculated with *S. aureus* (group S) had the highest TVB-N value, but the use of TP (0.2%, w/w) resulted in the maximum decrease in the value (group S-TP) following 9-d fermentation. This observation

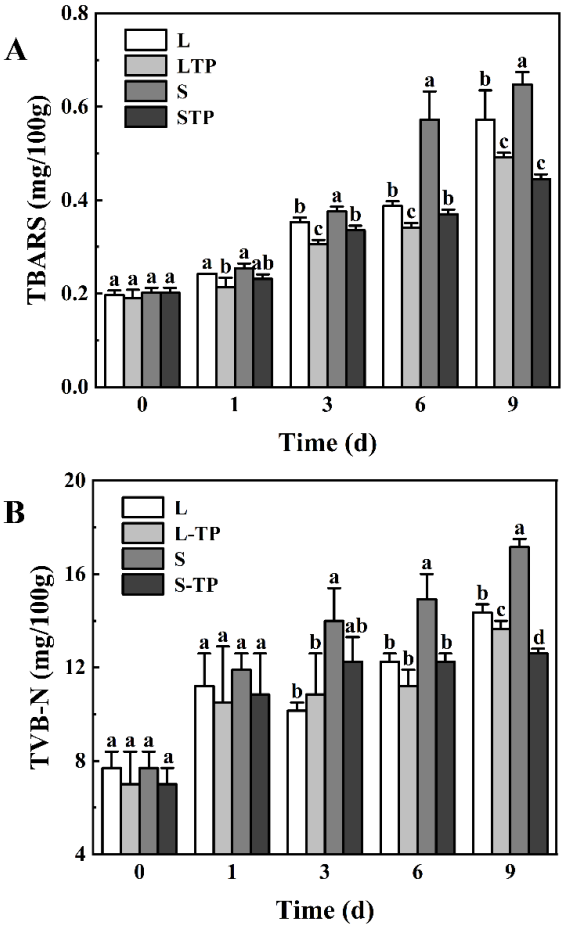

Figure 4. Effect of TP on TBARS **(A)** and TVB-N **(B)** values of dry fermented sausage inoculated with *L. plantarum* or *S. aureus*. Different letters on similar day indicate significant difference ($p < 0.05$).

indicated that TP could maintain the freshness of sausages by inhibiting *S. aureus* growth, even if the sausages were contaminated with this bacterium. In addition, TP could slow the increase in TVB-N through its antioxidant effect on lipids and proteins in sausages (Gonzalez-Burgos and Gomez-Serranillos, 2012). As a result of this two-fold influence, 0.2% (w/w) TP delayed the spoilage of sausages.

For all groups, the a_w of the sausages gradually decreased over time from an initial value of 0.778 to 0.652 owing to moisture loss, but there was no significant difference among the groups. Proper acidity is desirable for dry fermented sausages because it can inhibit the growth of harmful microorganisms, and contribute to product flavour (Laranjo *et al*., 2019). As shown in Table 1, the initial pH values of all sausage samples were approximately 5.9. In the case of groups L and L-TP, the pH value sharply decreased to approximately 5.4 during the first 6-d fermentation, and then remained stable. The

Table 1. Effect of TP on water activity (a_w) and pH of dry fermented sausage inoculated with *L. plantarum* or *S. aureus*.

Day	Group			
	L	L-TP	S	S-TP
			a_w	
0	0.75 ± 0.02^{Aa}	0.77 ± 0.01^{Aa}	0.75 ± 0.01^{Aa}	0.78 ± 0.01^{Aa}
1	0.74 ± 0.01^{ABb}	0.76 ± 0.02^{Aab}	0.74 ± 0.02^{Aab}	0.77 ± 0.02^{Aa}
3	0.72 ± 0.01^{Bb}	0.71 ± 0.01^{Bb}	0.74 ± 0.01^{Aa}	0.71 ± 0.01^{Bb}
6	0.69 ± 0.02^{Ca}	0.68 ± 0.01^{Ca}	0.69 ± 0.01^{Ba}	0.69 ± 0.03^{Ba}
9	0.65 ± 0.01^{Da}	0.66 ± 0.01^{Ca}	0.67 ± 0.02^{BCa}	0.68 ± 0.01^{BCa}
			pH	
0	5.89 ± 0.05^{Ab}	5.88 ± 0.02^{Abc}	5.97 ± 0.01^{Ba}	5.83 ± 0.03^{Bc}
1	5.68 ± 0.01^{Bc}	5.74 ± 0.01^{Bb}	5.99 ± 0.02^{Ba}	5.78 ± 0.01^{Cb}
3	5.47 ± 0.03^{Cc}	5.48 ± 0.04^{Cc}	6.04 ± 0.04^{Aa}	5.84 ± 0.01^{Bb}
6	5.42 ± 0.02^{Dc}	5.30 ± 0.02^{Dd}	5.96 ± 0.03^{Ba}	5.88 ± 0.03^{Ab}
9	5.42 ± 0.01^{Dc}	5.37 ± 0.01^{Ed}	5.94 ± 0.02^{Ba}	5.81 ± 0.01^{BCb}

L: *L. plantarum* inoculation 10^7 CFU/g; L-TP: L with 0.2% TP; S: *S. aureus* inoculation 10^7 CFU/g; and S-TP: S with 0.2% TP. Different uppercase superscripts in similar column indicate significant differences ($p < 0.05$); different lowercase superscripts in similar row indicate significant differences ($p < 0.05$).

pH of the L-TP group was significantly ($p < 0.01$) lower than that of group L after day 3. This finding agreed with a similar study which found that the use of natural polyphenols led to a pH decrease in fermented sausages (Zhang *et al*., 2017). Unlike groups L and L-TP, group S showed no obvious pH decrease following 9-d fermentation, but the pH of group S-TP was significantly ($p < 0.01$) lower than that of group S over time. This may be because the presence of TP promoted the growth of naturally occurring LAB in the sausages, thereby decreasing the pH value.

The lightness values of sausages decreased following 9-d fermentation, which was significantly correlated with the a_w of the sausages (Bozkurt and Bayram, 2006). For all groups, the sausages showed an increase in the *a** value, and a decrease in the *b** value following 9-d fermentation. Moreover, sausages with TP had higher *a** values than those without TP ($p < 0.05$). These observations suggested that TP could endow sausages with a more attractive colour while exerting dual-directional regulation on the growth of *L. plantarum* and *S. aureus*. Dong *et al*. (2019) indicated that this positive effect of polyphenols on meat colour is attributed to their ability to prevent myoglobin oxidation to form brown metmyoglobin. For the texture analysis, the fermentation of all sausage groups led to an increase in hardness, cohesiveness, and chewiness of products following 9-d fermentation, regardless of whether TP was used. Sausages with TP had lower hardness, cohesiveness, and chewiness values than those without TP, but there was no significant difference in most cases.

In conclusion, the addition of TP could regulate the growth of *L. plantarum* and *S. aureus* in sausages to improve biosafety, coupled with delaying lipid oxidation and spoilage without changing the textural characteristics.

Volatile compounds in dry fermented sausages

A total of 48 volatile compounds were identified in all the samples, including 11 esters, 13 aldehydes, seven alcohols, five ketones, and 11 terpenes. The L, L-TP, S, and S-TP groups contained 46, 39, 39, and 37 volatile compounds, respectively (Table 2). Ethyl esters are essential for the characteristic aroma of dry sausages because they impart fruity, sweet, and floral notes to the product due to their low odour threshold values (Sidira *et al*., 2015). In the present work, L-TP exhibited the highest total ester content. Furthermore, the contents of most ethyl esters in the L-TP and S-TP sausages were significantly higher than those in the sausages without TP ($p < 0.05$), such as ethyl hexanoate (pineapple), ethyl heptanoate (pineapple and wine), ethyl dihydrocinnamate (fruit and honey), and ethyl hexadecanoate (wax, fruit, and cream). Previous

Table 2. Effect of TP on volatile compounds of dry fermented sausage inoculated with *L. plantarum* or *S. aureus*.

Volatile component (μg/kg)	L	L-TP	S	S-TP
Ester				
Ethyl hexanoate	135.21 ± 11.40^{b}	240.60 ± 24.87^{a}	85.80 ± 1.04^{c}	112.45 ± 7.81^{b}
Ethyl heptanoate	0.27 ± 0.16^{c}	1.47 ± 0.21^{a}	0.88 ± 1.04^{b}	1.46 ± 0.10^{a}
Ethyl nonanoate	3.74 ± 1.09^{b}	3.39 ± 0.43^{b}	2.99 ± 0.20^{b}	4.90 ± 0.48^{a}
Terpinyl acetate	3.12 ± 1.99^{a}	2.92 ± 0.19^{a}	1.67 ± 0.06^{b}	2.57 ± 0.39^{ab}
Ethyl dihydrocinnamate	1.13 ± 0.13^{c}	14.11 ± 2.28^{a}	2.32 ± 0.39^{c}	5.60 ± 0.99^{b}
Ethyl decanoate	1.76 ± 0.35^{b}	25.68 ± 3.28^{a}	1.64 ± 0.24^{b}	2.19 ± 0.21^{b}
Ethyl dodecanoate	0.63 ± 0.13^{b}	1.00 ± 0.28^{a}	0.66 ± 0.18^{b}	ND
Ethyl tetradecanoate	0.88 ± 0.33^{c}	2.18 ± 0.46^{a}	1.17 ± 0.18^{bc}	1.65 ± 0.22^{ab}
Ethyl hexadecanoate	2.14 ± 0.62^{b}	5.19 ± 0.98^{a}	3.02 ± 0.24^{b}	4.15 ± 0.35^{a}
Linalyl acetate	0.55 ± 0.18^{b}	1.03 ± 0.21^{a}	0.51 ± 0.10^{b}	1.04 ± 0.15^{a}
Ethyl lactate	ND	28.20 ± 8.0^{9}	ND	ND
Total	149.45 ± 14.01^{b}	325.78 ± 39.34^{a}	100.66 ± 1.97^{c}	136.02 ± 8.07^{b}
Aldehyde				
Hexanal	121.45 ± 14.80^{a}	8.56 ± 1.02^{c}	24.88 ± 0.47^{b}	3.45 ± 1.18^{c}
Nonanal	29.29 ± 5.92^{b}	49.22 ± 3.48^{a}	21.09 ± 0.41^{c}	34.09 ± 1.93^{b}
(*E*)-2-Nonenal	7.98 ± 1.67^{a}	2.26 ± 0.23^{b}	1.91 ± 0.33^{b}	1.12 ± 0.37^{c}
Decanal	0.60 ± 0.24^{b}	1.22 ± 0.44^{a}	0.59 ± 0.11^{b}	1.07 ± 0.23^{ab}
p-Isopropylbenzaldehyde	2.84 ± 0.38^{d}	9.43 ± 1.66^{b}	5.41 ± 0.22^{c}	12.13 ± 0.34^{a}
Heptanal	5.88 ± 2.40	ND	ND	ND
(*Z*)-2-Heptenal	33.64 ± 2.80^{a}	ND	3.80 ± 0.89^{b}	ND
(*E*)-2-Decenal	6.85 ± 2.67^{a}	0.98 ± 0.29^{b}	1.77 ± 0.39^{b}	ND
(*E*)-2-Octenal	14.77 ± 5.38	ND	ND	ND
(*E, E*)-2,4-Nonadienal	3.95 ± 0.77	ND	ND	ND
(*E, E*)-2,4-Decadienal	3.12 ± 1.99	ND	ND	ND
Tetradecanal	2.01 ± 0.69	ND	ND	ND
Benzeneacetaldehyde	ND	7.55 ± 2.18^{a}	ND	8.96 ± 0.27^{a}
Total	232.37 ± 37.10^{a}	79.22 ± 9.08^{b}	59.45 ± 1.26^{b}	60.84 ± 2.23^{b}
Alcohol				
Ethanol	312.30 ± 28.58^{c}	652.06 ± 38.58^{a}	416.27 ± 52.30^{b}	595.25 ± 23.89^{a}
Linalool	4.63 ± 0.32^{c}	8.57 ± 0.90^{a}	6.04 ± 0.28^{b}	8.81 ± 0.97^{a}
Isoborneol	1.07 ± 0.23^{c}	2.68 ± 0.79^{a}	1.05 ± 0.22^{c}	2.01 ± 0.17^{b}
1-Octen-3-ol	11.33 ± 2.16^{a}	ND	2.40 ± 0.82^{b}	0.72 ± 0.08^{c}
1-Octanol	1.43 ± 0.39	ND	ND	ND
Terpinen-4-ol	2.47 ± 0.23^{b}	5.67 ± 1.14^{a}	2.90 ± 0.12^{b}	4.83 ± 0.54^{a}
α-Terpineol	1.76 ± 0.01^{a}	3.46 ± 0.59^{b}	2.00 ± 0.13^{a}	2.80 ± 0.67^{b}
Total	335.06 ± 29.01^{c}	672.45 ± 40.39^{a}	430.66 ± 51.81^{b}	614.43 ± 23.31^{a}
Ketone				
Fenchone	1.83 ± 0.60^{b}	3.86 ± 0.57^{a}	2.31 ± 0.28^{b}	4.16 ± 0.61^{a}
2-Nonanone	0.92 ± 0.15^{b}	2.02 ± 0.22^{a}	0.87 ± 0.17^{b}	2.00 ± 0.29^{a}
2-Undecanone	0.65 ± 0.25^{b}	2.23 ± 0.75^{a}	0.95 ± 0.30^{b}	2.20 ± 0.42^{a}
Piperitone	2.83 ± 0.43^{b}	5.76 ± 0.96^{a}	3.26 ± 0.22^{b}	5.72 ± 0.53^{a}

2, 3-Octanedione	25.95 ± 4.57	ND	ND	ND
Total	32.18 ± 4.81^{a}	13.87 ± 2.28^{b}	7.37 ± 0.71^{c}	14.08 ± 0.95^{b}
Terpene				
D-Limonene	41.78 ± 3.00^{c}	81.97 ± 6.42^{a}	46.31 ± 2.33^{c}	63.26 ± 4.32^{b}
Copaene	13.79 ± 1.02^{c}	31.48 ± 3.34^{a}	18.03 ± 0.95^{b}	29.00 ± 1.57^{a}
Caryophyllene	1.53 ± 0.21^{d}	3.78 ± 0.44^{b}	2.49 ± 0.10^{c}	4.76 ± 0.40^{a}
α-Bergamotene	1.41 ± 0.07^{d}	3.15 ± 0.22^{b}	2.01 ± 0.33^{c}	4.23 ± 0.23^{a}
α-Curcumene	2.87 ± 0.30^{b}	6.40 ± 0.82^{a}	3.63 ± 0.17^{b}	6.06 ± 0.51^{a}
Zingiberene	0.70 ± 0.05^{c}	4.27 ± 0.77^{a}	2.16 ± 0.19^{b}	4.98 ± 1.11^{a}
β-Bisabolene	1.80 ± 0.33^{d}	4.17 ± 0.40^{b}	2.80 ± 0.06^{c}	5.45 ± 0.41^{a}
Cadina-1(10),4-diene	0.82 ± 0.13^{b}	2.64 ± 0.31^{a}	1.20 ± 0.22^{b}	2.72 ± 0.53^{a}
Estragole	102.32 ± 1.31^{c}	265.12 ± 24.83^{a}	147.92 ± 5.36^{b}	244.15 ± 17.92^{a}
β-Sesquiphellandrene	1.12 ± 0.26^{b}	3.85 ± 0.86^{a}	1.75 ± 0.04^{b}	3.84 ± 0.71^{a}
γ-Muurolene	0.57 ± 0.13^{c}	1.75 ± 0.05^{a}	0.87 ± 0.09^{b}	1.16 ± 0.32^{b}
Total	66.39 ± 4.63^{d}	143.48 ± 12.83^{a}	81.25 ± 3.04^{c}	125.46 ± 4.37^{b}
Other				
Ethyl (*Z*)-4-decenoate	0.56 ± 0.08^{abc}	0.88 ± 0.08^{a}	0.49 ± 0.06^{c}	0.64 ± 0.26^{abc}

L: *L. plantarum* inoculation 10^7 CFU/g; L-TP: L with 0.2% TP; S: *S. aureus* inoculation 10^7 CFU/g; and S-TP: S with 0.2% TP. Different lowercase superscripts in similar row indicate significant differences ($p < 0.05$). ND: not detected.

studies have shown that LAB could significantly promote the formation of ester volatile compounds, therefore, the probiotic effect of TP may favour the accumulation of ethyl esters (Zhou *et al*., 2021).

Aldehydes are typical products of lipid oxidation that exhibit low odour thresholds and distinctive odour characteristics. Nonanal imparts a citrus odour, benzeneacetaldehyde imparts a typical mushroom odour, and high amounts of hexanal generally result in a rancid flavour (Xiao *et al*., 2020). As shown in Table 2, the samples treated with TP showed higher nonanal and benzeneacetaldehyde contents, suggesting that TP imparted stronger pleasant flavour. The lower hexanal and total aldehyde contents in the L-TP and P-TP samples indicated that lipid oxidation was inhibited by TP. Similarly, Dai *et al*. (2022) found that a chitosan-EGCG coating was effective in inhibiting the accumulation of aldehydes in fish, which might be due to the ability of polyphenols to scavenge free radicals, chelate free iron, and quench free radical chain reactions.

The main sources of alcohol are microbial carbohydrate metabolism, lipid oxidation, and amino acid decarboxylation (Li *et al*., 2023). Ethanol showed the highest proportion in this category, but it usually contributes less to the overall aroma of fermented sausages owing to its high odour threshold. Linalool was found to have strong floral and fruity aromas, and higher levels of linalool were detected in the L-TP and P-TP groups. 1-Octanol, which contributes to fatty and citrus flavours, was only detected in the L group. Ketones, formed from the degradation of amino acids, fat oxidation, and microbial catabolism, may also be of great importance to sausage aroma due to their contribution to the formation of milk flavour (Hu *et al*., 2022). In the present work, sausages treated with TP contained higher levels of most ketone compounds (fenchone, 2-nonanone, 2-undecanone, and piperitone) compared to those without. In addition, 11 terpenes, which made up a significant proportion of the overall composition, were identified in all samples; However, they were not considered essential to the overall flavour due to their high odour thresholds (Zhang *et al*., 2018). Collectively, these results suggested that the addition of TP improved flavour development in dry fermented sausages.

Conclusion

At appropriate concentrations, TP showed a dual-directional regulation on the growth of probiotic *L. plantarum*, pathogenic *S. aureus*, and *E. coli*. It could boost the proliferation of *L. plantarum* while inhibiting *S. aureus* and *E. coli* during co-culture.

This dual-directional regulatory action of TP remained effective during the production of dry-fermented sausages, where *L. plantarum* multiplied well, while *S. aureus* was considerably suppressed in the presence of TP. Moreover, TP effectively reduced TBARS and TVB-N values during sausage maturation, and improved the product quality and flavour to a certain extent. These results would be significant for the application of TP in fermented meats, especially in naturally fermented meats, considering product quality and safety.

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