Food Science of Animal Products

Research Article

Preparation of astaxanthin-rich nanoparticulate fermented duck sausages with nitrite-reducing *Lactobacillus*: enhanced antioxidant capacity and reduced nitrite

Xiaozhi Wang[1,2,§], Ping Ye[1,§], Xiaoyu Xu[1], Yage Wang[1], Zi'ang Li[1], Weimei Kong[1], Haibo Luo[1], Mingxuan Tao[1], Chen Liu[1], Xiaoqun Zeng[3], Zhen Wu[3], Daodong Pan[3], Yuxing Guo[1] ✉

[1] *Department of Food Science and Technology, School of Food Science and Pharmaceutical Engineering, Nanjing Normal University, Nanjing 210023, China*
[2] *Wen's Food Group Co., Ltd., Yunfu 527300, China*
[3] *State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Key Laboratory of Animal Protein Food Processing Technology of Zhejiang Province, Ningbo University, Ningbo 315211, China*
[§] These authors contributed equally to this article.
✉ Address correspondence to Yuxing Guo, guoyuxing1981@163.com

Received: December 26, 2025; Revised: January 13, 2026; Accepted: February 27, 2026

Abstract: Nitrite is a major hazardous substance in fermented sausages, and excessive human intake poses health risks. This study aimed to develop a low-nitrite fermented duck sausage. Lactic acid bacteria (LAB) can effectively degrade nitrite; astaxanthin exhibits excellent antioxidant and colorant properties, and nanoencapsulation technology can improve astaxanthin stability. Additionally, milk fat globule membrane-enriched whey protein powder (MFGM-WPI) is a promising food-grade encapsulation wall material. In this study, a selected strain of *Lactobacillus sakei* was combined with other LAB as the starter culture for duck sausage fermentation. Furthermore, gum arabic and MFGM-WPI were used as raw materials to prepare a novel astaxanthin nanoparticle additive, which aimed to improve the color of the sausage and endow it with antioxidant effects. The results showed that the fermented duck sausages added with astaxanthin-rich nanoparticles and composite LAB exhibited superior color stability and textural properties, characterized by a reddish hue, reduced nitrite content, and enhanced astaxanthin levels. Among all tested fermented duck sausage groups (including the natural fermentation group, single LAB fermentation group, and free astaxanthin-added group), the specific group (astaxanthin-rich nanoparticles + composite LAB) exhibited the highest 1,1-diphenyl-2-picrylhydrazyl radical and hydroxyl radical scavenging activities, alongside the lowest peroxide value. In conclusion, the combination of composite LAB strains and astaxanthin nanoparticles represents a potentially effective strategy for reducing nitrite content and enhancing the antioxidant capacity of fermented duck sausages.

Keywords: *Lactobacillus sakei*; nitrite reduction; astaxanthin nanoparticles; milk fat globule membranes; fermented duck sausages

Citation: X. Z. Wang, P. Ye, X. Y. Xu, et al., Preparation of astaxanthin-rich nanoparticulate fermented duck sausages with nitrite-reducing *Lactobacillus*: enhanced antioxidant capacity and reduced nitrite, Food Sci. Anim. Prod. 4 (2026) 9240162. https://doi.org/10.26599/FSAP.2026.9240162.

1 Introduction

Nitrite plays a crucial role as a food additive in meat products, with its core functions being to impart unique flavor and characteristic color to meat products, while inhibiting the growth and reproduction of most pathogenic bacteria and spoilage microorganisms to ensure the product's shelf life[1–2]. However, if the human body ingests nitrite for a long time and excessive accumulation occurs in the body, it will cause irreparable harm to health. For instance, the strong oxidizing property of nitrite promotes the conversion of human hemoglobin into methemoglobin, which cannot bind oxygen normally. This ultimately leads to a significant decrease in the oxygen-carrying capacity of the blood, and in severe cases, can cause tissue hypoxia[3]. Nitrite can be substituted by other coloring agents[4] and degraded by microorganisms, particularly lactic acid bacteria (LAB)[5]. Fermented sausages, a meat product, can reduce the harm of nitrite by LAB. To ensure the quality of fermented sausages, needs to choose nitrite alternatives with excellent color and antioxidant properties[6]. Thus, screening and isolation of excellent LAB and finding nitrite substitutes to play a role in the fermentation process of meat products is a very vital mission, and it's crucial to find non-toxic, secure substitutes for nitrite.

Astaxanthin is a fat-soluble carotenoid with excellent antioxidant activities, which has been widely used in the fields of food and medicine due to its natural colouring properties[7–8]. Therefore, the commercial application of astaxanthin as a supplement in food and diet has been increasing in recent years. However, astaxanthin is unstable and insoluble in water under conditions such as light and heat[9], which requires effective strategies to improve its stability and bioavailability, including emulsion, liposome, nanodispersion, and microencapsulation techniques[10–12]. Nanoencapsulations possess a greater surface area per unit volume, resulting in increased solubility and greatly enhanced bioactivity. The size of the prepared capsule will affect the targeted delivery of the bioactive substance in

the body. Therefore, nanoencapsulations have advantages over conventional microcapsules in terms of improved bioavailability of bioactives, effective controlled release, and precise targeting[13].

As usual, astaxanthin is more bioavailable when taken with proteins or fat-rich foods such as milk[14–15]. Food-grade proteins have been widely used to improve the bioavailability of fat-soluble active substances, for instance, milk fat globule membrane (MFGM) proteins[16], β-lactoglobulin, soy protein, gelatin, and casein, due to their natural origin, non-toxic nature, and high nutritional value[17]. Milk, as a fat-rich raw material, contains a variety of milk proteins, including casein, whey, and lactoferrin, which mitigate the oxidation and degradation of astaxanthin as antioxidants in emulsions and dispersion systems, thus enhancing the stability of astaxanthin to a large extent[18]. There are many different types of whey protein powders, of which MFGM-enriched whey protein powder (MFGM-WPI) is a new type of dairy ingredient. Currently, MFGM-WPI is often used not only as an additive for infants, but is also likely to be used as antioxidants in cooking oils and as active ingredients for functional foods[19], but it has not yet been studied as an encapsulated wall material. Therefore, in this study, MFGM-WPI was used as a wall material to encapsulate astaxanthin to make astaxanthin nanoparticles as a raw material for experiments.

In the current study, LAB capable of efficient nitrate reduction were screened from traditional fermented foods, with the aim of evaluating their potential as novel starter cultures for fermented duck sausage production. These selected LAB strains were further combined with astaxanthin nanoparticles to develop an innovative fermentation system. Specifically, MFGM-gum arabic (GA)-astaxanthin nanoparticle complexes were investigated as partial nitrite substitutes, with particular focus on their dual functionality in color enhancement and antioxidant delivery.

2 Materials and methods

2.1 Materials

Duck breasts, pork back fat, salt, cellulose casing, and sugar powder were purchased from a local market (Nanjing, China). MFGM-WPI (protein 70.0%, phospholipid 6.5%, fat 15.0%, lactose 3.0%–6.0%, moisture 4.5%, and ash 3.0%, pH 7.0) was purchased from Hilmar Ingredients (Hilmar, CA, USA). GA was offered by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Nitrite was purchased from Sichuan Jinshan Pharmaceutical Co., Ltd. (Meishan, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activity kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). *Lactobacillus helveticus* CGMCC 24356, *Lactobacillus casei* CGMCC 15956, *Lactobacillus bulgaricus* CGMCC 21396, *Lactobacillus acidophilus* CICC 6074, and *Lactiplantibacillus plantarum* CGMCC 23701 were isolated and preserved in our laboratory (Nanjing Normal University, Nanjing, China).

2.2 Screening of nitrite-reducing LAB *in vitro*

A total of five LAB strains, isolated from the salted fish, kimchi, and sausages, were screened for their potential probiotic properties. The LAB strains were cultured at 37 °C for 32 h in Man-Rogosa-Sharpe (MRS) broth (Oxoid, Basingstoke, UK). Then, the strains were inoculated (1.0%, *V*/*V*) in MRS broth containing 150 mg/L nitrite, and the nitrite degradation capacity of LAB was estimated by detecting changes in nitrite levels at 0, 6, 12, 18, and 24 h. Nitrite content in the sausages was evaluated by the spectrophotometric method in accordance with the national standard method (GB 5009.33–2016) in China, and the degradation rate of nitrite was calculated according to formula (1):

$$\text{Degradation rate of nitrite}\,(\%) = \frac{\rho_0 - \rho_1}{\rho_0} \times 100 \quad (1)$$

Where ρ_0 was the initial content of nitrite in the culture medium (mg/L), and ρ_1 was the nitrite content in the medium at different incubation moments (mg/L).

Furthermore, we determined the degradation rate of nitrite of *L. helveticus* CGMCC 24356, *L. casei* CGMCC 15956, *L. bulgaricus* CGMCC 21396, *L. acidophilus* CICC 6074, and *L. plantarum* CGMCC 23701 using the previously mentioned methodology.

2.3 Characterization of LAB strains

2.3.1 Strain identification

The isolate that showed the strongest nitrite-reducing activity was subjected to biochemical identification by 16S rDNA gene sequencing analysis[20]. The total DNA of the strain was extracted. The strain's 16S rDNA gene was amplified using polymerase chain reaction (PCR) with primers 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) and 27F (5′-AGAGTTTGATCMTGGCTCAG-3′), which were purchased from Sangon Biotech (Shanghai, China). The fragment (1 500 bp) was purified using the rapid PCR purification kit (Takara, Kyoto, Japan) and sequenced by Sangon Biotech (Shanghai, China). A similarity-based search of the quality control database of 16S rRNA sequences was performed using the identification web server of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using FastTree v2.1.11 and the generalized time-reversible and GAMMA models. Support values were determined using 100 nonparametric bootstrapping. The tree was visualized using iToL and formatted using Inkscape 1.1 (http://inkscape.org). In the end, the identified strain was deposited in the China General Microbiological Culture Collection Center (CGMCC; Beijing, China) with ID CGMCC 23702.

2.3.2 Growth curve determination

The LAB were streaked onto MRS agar medium under aseptic conditions and incubated statically at 37 °C until single colonies emerged. Single colonies were inoculated into MRS liquid medium and incubated statically at 37 °C for two cycles of activation. After being homogenized, the culture was inoculated into MRS liquid medium at a volume ratio of 1.0%, followed by incubation at 37 °C for 24 h. The culture broth was sampled every 3 h, and the $OD_{600\,nm}$ value was determined by turbidimetry to plot the growth curve.

2.3.3 Simulated gastric and intestinal fluid tolerance determination

Simulated gastric fluid (SGF) was prepared with 0.5 g/100 mL NaCl and 0.35 g/100 mL pepsin, adjusted to pH 2.5 with 1 mol/L HCl, and then filtered and sterilized through a 0.22 μm membrane filter. Simulated intestinal fluid (SIF) was prepared with 0.5 g/100 mL NaCl and 0.1 g/100 mL trypsin, adjusted to pH 8.0 with 1 mol/L NaOH, followed by filtration and sterilization through a 0.22 μm membrane filter. The activated strain culture was inoculated into SGF at a volume ratio of 1:10 and incubated at 37 °C with shaking at 120 r/min for 2 h. After incubation, the viable cell count was determined by the plate counting method. For the SIF tolerance

assay, the activated culture was inoculated into SIF at 1:10 (*V*/*V*) and incubated at 37 °C with shaking at 120 r/min for 6 h, and the viable cell count was then measured. The survival rate was calculated using the following formula (2):

$$\text{Survival rate}\ (\%) = \frac{\lg N_1}{\lg N_0} \times 100 \quad (2)$$

Where N_1 is the number of viable bacteria under different treatment conditions, and N_0 is the number of viable bacteria in the normal MRS medium.

2.3.4 Acid and bile tolerance determination

LAB cells were collected by centrifugation at 3 000 × *g* for 10 min after growth in MRS broth at 37 °C. The collected cells were suspended in MRS broth at different pH values (2.0, 3.0, 4.0, and 5.0) and bile salt concentrations (0, 0.05, 0.10, 0.20, and 0.30 g/100 mL) and incubated for 3 h at 37 °C. The viable cell count was determined through plate counting every hour, and the survival rate was calculated using formula (2).

2.3.5 Antibacterial activity determination

Escherichia coli and *Staphylococcus aureus* were used as indicator strains, cultured overnight in Luria-Bertani (LB) medium, and then spread on LB agar plates. *Lactobacillus* strains were inoculated into MRS broth using an inoculation loop and cultured for 48–72 h. The culture was centrifuged at 8 000 × *g* for 10 min at 4 °C in a high-speed refrigerated centrifuge, and the supernatant was filtered through a 0.22 μm filter membrane. Using the Oxford cup method, wells were punched on the agar plates, and 200 μL of fermentation supernatant was added into each well. After incubation at 37 ℃ for 24 h, the antibacterial activity was determined by measuring the diameter of the inhibition zones.

2.4 Preparation of fermented duck sausage

The sausage formulation (1 kg per batch) included 80% duck breasts and 20% pork back fat. The meat was mixed with 4% (*m*/*m*) sodium chloride, 3.2% (*m*/*m*) glucose, 3.5% (*m*/*m*) cooking wine, 0.4% (*m*/*m*) white pepper, 0.015% (*m*/*m*) nitrite, 0.1% (*m*/*m*) compound phosphates, 6% (*m*/*m*) strains, all based on the total mass of duck breasts and pork back fat. After chopping and mixing the ingredients, the fermented duck sausages were divided into three groups (10 kg per group): 1) Control (natural fermentation), 2) Ls (*L. sakei*), and 3) LAB (*L. sakei*, *L. helveticus*, *L. casei*, *L. bulgaricus*, *L. acidophilus*, and *L. plantarum* were mixed at a volume ratio of 1:1:1:1:1:1), to explore the excellent performance of the LAB fermentation. The sausage mixture was stuffed into a cellulose casing (3.8 cm diameter) and then placed in a fermentation chamber. The fermentation and ripening program was set as follows: fermentation at 30 °C for 7 days, with a relative humidity of (85 ± 3)%, and subsequent dehydration at 50 °C for 4 h. Triplicate samples were collected from each group on fermentation days 1, 3, 5, and 7 for microbiological, textural, and physicochemical analysis.

2.5 Preparation of astaxanthin nanoparticles

The free astaxanthin was extracted from *Haematococcus pluvialis* according to a previously published method from our research group[21]. Briefly, *H. pluvialis* was pretreated with 0.2 mol/L acetic acid-sodium acetate buffer (pH 6.0) at a solid-liquid ratio of 20:1 (mg/mL), followed by the addition of 0.4 mg/mL snailase (Aladdin, Shanghai, China) and incubation at 35 °C for 4 h. The residue was collected and extracted with anhydrous ethanol at a solid-liquid ratio of 5:1 (mg/mL) in a 50 °C water bath for 1 h, and the extract was filtered through a 0.45 μm syringe filter.

Astaxanthin nanoparticles were prepared using MFGM-GA as the carrier. The protein-polysaccharide complex was prepared with a mass ratio of MFGM-WPI to GA of 2:1 at pH 4.0, and the total carrier concentration was fixed at 0.15 g/100 mL. Astaxanthin (60 μmol/L) was added to the MFGM-GA solution, and the mixture was stirred at 500 r/min for 1 h in the dark to obtain the final astaxanthin nanoparticles.

2.6 Fluorescence spectra analysis

The interaction between MFGM proteins and astaxanthin was analyzed by fluorescence spectroscopy using an F380 fluorescence spectrophotometer (Gangdong Technology Co., Ltd., Tianjin, China). All samples were measured at an excitation wavelength of 280 nm, with excitation and emission slits both set to 5 nm. Emission spectra were recorded from 300 to 450 nm at a medium voltage (600 V)[11].

2.7 Preparation of fermented duck sausages with astaxanthin nanoparticles

Four treatment groups (10 kg per group) of fermented duck sausages were prepared to investigate the effect of astaxanthin nanoparticles on product quality. The four groups were designed as follows: 1) FA (free astaxanthin), 2) NA (astaxanthin nanoparticles), 3) FAL (free astaxanthin + LAB), and 4) NAL (astaxanthin nanoparticles + LAB). The starter culture was all compound LAB (6%), and the addition of free astaxanthin and astaxanthin nanoparticles was 2% (*m*/*m*), respectively. The other procedures were performed according to section 2.4.

2.8 Microbiological and physicochemical analysis

2.8.1 Microbiological analysis

After fermentation and drying, sterile samples were taken from the center of sausages in different groups for viable bacteria count determination, with slight modifications based on the method described by Liu et al.[22]. Samples (10 g each) for every formulation were homogenized in 90 mL of sterile saline (0.91% (*m*/*m*) NaCl) after being aseptically extracted from the core of different groups of sausages. Serial 10-fold dilutions were prepared and spread-plate in duplicate on MRS agar in anaerobic conditions after 48 h at 37 °C.

2.8.2 Physicochemical analysis

An appropriate amount of fermented sausage samples was taken, diluted with distilled water, and thoroughly stirred to form a homogeneous suspension, the pH value of the suspension was measured using a FE28-Micro pH meter (Mettler Toledo, Greifensee, Switzerland).

The redness value (a^*) of fermented sausages was determined using a colorimeter (WSC-S, Shanghai, China) based on the $L^*a^*b^*$ color space system.

2.9 Texture profile analysis

A CT-3 type texture analyzer (Brookfield, Middleboro, MA, USA) were used to determine the texture characteristics of fermented sausages at room temperature. After removing the sausage casing,

the sausages were cut into uniform slices (10 mm thickness). The test parameters were set as follows: TA39 probe with a diameter of 50 mm, 50% strain, test speed of 5 mm/s, and a 5 s interval between two consecutive measurements. The main texture parameters measured included hardness, elasticity, chewiness, and adhesiveness.

2.10 Astaxanthin retention rate determination

The 1 g sample of the fermented sausage was mixed with 5 mL of anhydrous ethanol, homogenized into a slurry, and centrifuged at 10 000 × *g* for 10 min to collect the supernatant. Repeat the operation until the supernatant is colorless and measure the absorbance at 476 nm. The astaxanthin rentention rate was calculated according to formula (3)[23]:

$$\text{Axanthin rentention rate}\,(\%) = \frac{V \times \rho_2}{m} \times 100 \quad (3)$$

Where V indicated the volume of the supernatant after combining (mL); ρ_2 indicated the concentration of astaxanthin (μg/mL); m indicated the initial mass of astaxanthin used (mg).

2.11 Antioxidant propertity determination

For DPPH radical scavenging activity, 2.0 mL of sample solution from different groups was mixed with 2.0 mL of 0.1 mmol/L DPPH solution (dissolved in 95% (*V*/*V*) ethanol). The mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm[24]. The DPPH radical scavenging rate was calculated according to formula (4):

$$\text{Scavenging rate}\,(\%) = \frac{A_0 - A_x}{A_0} \times 100 \quad (4)$$

Where A_x was the absorbance of the sample solution mixed with the DPPH solution, and A_0 was the absorbance of 2 mL of DPPH solution.

For hydroxyl radical scavenging activity, 2.0 mL of sample was mixed with 2.0 mL of 6 mmol/L $FeSO_4$, 2.0 mL of 6 mmol/L salicylic acid-ethanol, and 2.0 mL of 6 mmol/L H_2O_2. After incubation at 37 °C for 30 min, absorbance was recorded at 510 nm. The hydroxyl radical scavenging rate was calculated according to formula (4). Where A_x was the absorbance of the sample and A_0 was the absorbance of the control (distilled water instead of sample).

Peroxide value (POV) was determined according to a previously reported method[25]. In brief, the sample was mixed with a chloroform/glacial acetic acid mixture and potassium iodide solution, and incubated in the dark. The liberated iodine was titrated with standardized sodium thiosulfate solution.

2.12 Statistical analysis

All experiments were performed in triplicate and the results were expressed as mean ± standard deviation. Data were submitted for an analysis of variance (ANOVA). Differences between the means were verified by Tukey's test ($P < 0.05$). Data analysis and graphing were performed using GraphPad Prism 8 (GraphPad, San Diego, CA, USA) and IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA).

3 Results

3.1 An isolate of *L. sakei* Y1 was identified to display nitrite-reducing activity

Five LAB strains (P1, P2, Y1, Y2, and C) were screened from 50 candidate strains, and nitrite reduction activity was used as the key criterion for strain selection. Strain Y1, which was isolated from salted fish, showed the highest nitrite degradation rate of (92.16 ± 1.97)% and was selected as the preferred fermentation strain (Figure 1A). Cluster analysis showed that the identified strain was clustered into the *Lactobacillus* branch with high similarity, which was consistent with the 16S rRNA homology alignment results. Strain Y1 exhibited the highest homology (99%) with the type strain of *L. sakei* (Figure 1B). It was deposited at CGMCC with accession number CGMCC 23702 and identified as *L. sakei*

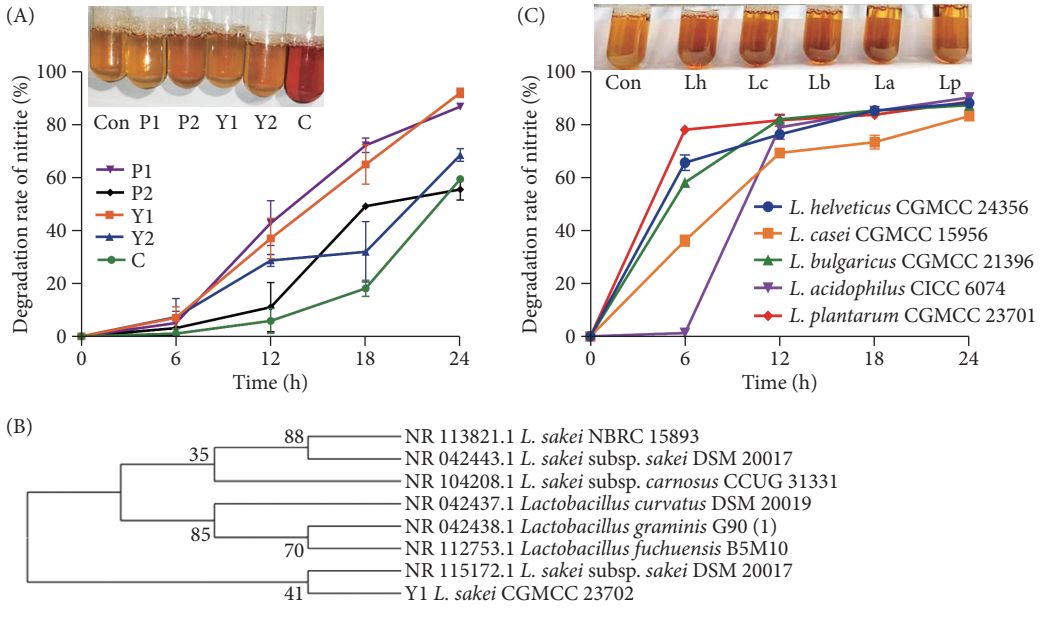

Figure 1 (A) Nitrite degradation rates of strains P1, P2, Y1, Y2, and C within 24 h; (B) phylogenetic tree of strain Y1 based on 16S rRNA gene sequence, constructed using MEGA software; (C) nitrite degradation rates of *L. helveticus*, *L. casei*, *L. bulgaricus*, *L. acidophilus*, and *L. plantarum* within 24 h. Insets show the corresponding *N*-(1-naphthyl)ethylenediamine dihydrochloride color development reactions. Con indicates the control group (blank MRS solution), while Lh, Lc, Lb, La, and Lp represent *L. helveticus*, *L. casei*, *L. bulgaricus*, *L. acidophilus*, and *L. plantarum*, respectively.

CGMCC 23702. Furthermore, the nitrite degradation rates of different LAB strains within 24 h were compared. *L. sakei* CGMCC 23702 showed better nitrite-reducing capacity than *L. acidophilus* ((90.10 ± 0.80)%), *L. casei* ((83.11 ± 1.47)%), *L. plantarum* ((88.53 ± 1.60)%), *L. helveticus* ((88.01 ± 1.62)%), and *L. bulgaricus* ((87.24 ± 1.29)%) (Figure 1C).

As illustrated in Figure 2A, the growth curve of LAB followed an upward tendency. Concretely, the colony count rose sharply following an interval of slow growth and plateaued at 18 h. The acid production curve of *L. sakei* (Figure 2B) exhibited an incremental level, where the acid production rate progressively increased after a 9-h depression. *L. sakei* demonstrated outstanding tolerance under diverse environments, as the survival rate reached (79.15 ± 1.24)% after 2-h incubation of gastric fluid (Figure 2C), (87.61 ± 0.17)% after 6-h incubation of artificial intestinal fluid (Figure 2D), and (57.48 ± 0.61)% under a pH 3 environment (Figure 2E). It was observed that *L. sakei* maintained a satisfactory survival rate of (54.23 ± 0.21)% under an environment with the bile salt concentration up to 0.30 g/100 mL, despite the decreasing survival rate with increasing bile salt concentration ranging from 0.03 to 0.30 g/100 mL (Figure 2F). Moreover, we concluded that *L. sakei* had an inhibitory effect on the growth of *E. coli* and *S. aureus* for the considerable diameter of the inhibition circles, the value of which was (11.02 ± 0.18) and (8.16 ± 0.28) mm, respectively (Figure 2G).

3.2 Performance comparison of various fermenters for fermented sausage

The LAB population in the Ls and LAB groups was higher than the control group ($P < 0.05$), indicating that LAB inoculated into the fermented sausages grew well within 48 h (Figure 3A). It is possible to quickly lower the sausage pH and produce good acidity to ensure rapid production of the special flavor of the sausage by adding LAB as a starter culture. The results showed that both Ls and LAB groups significantly reduced the pH of the sausages during the fermentation process ($P < 0.05$, Figure 3B). The nitrite content of fermented sausages in the Ls and LAB groups was lower than that of fermented sausages in the control group, and the LAB group had the lowest nitrite content of fermented sausages (Figure 3C). The a^* of the LAB group was significantly lower than that of the control or Ls groups as the sausage fermentation time increased ($P < 0.05$, Figure 3D).

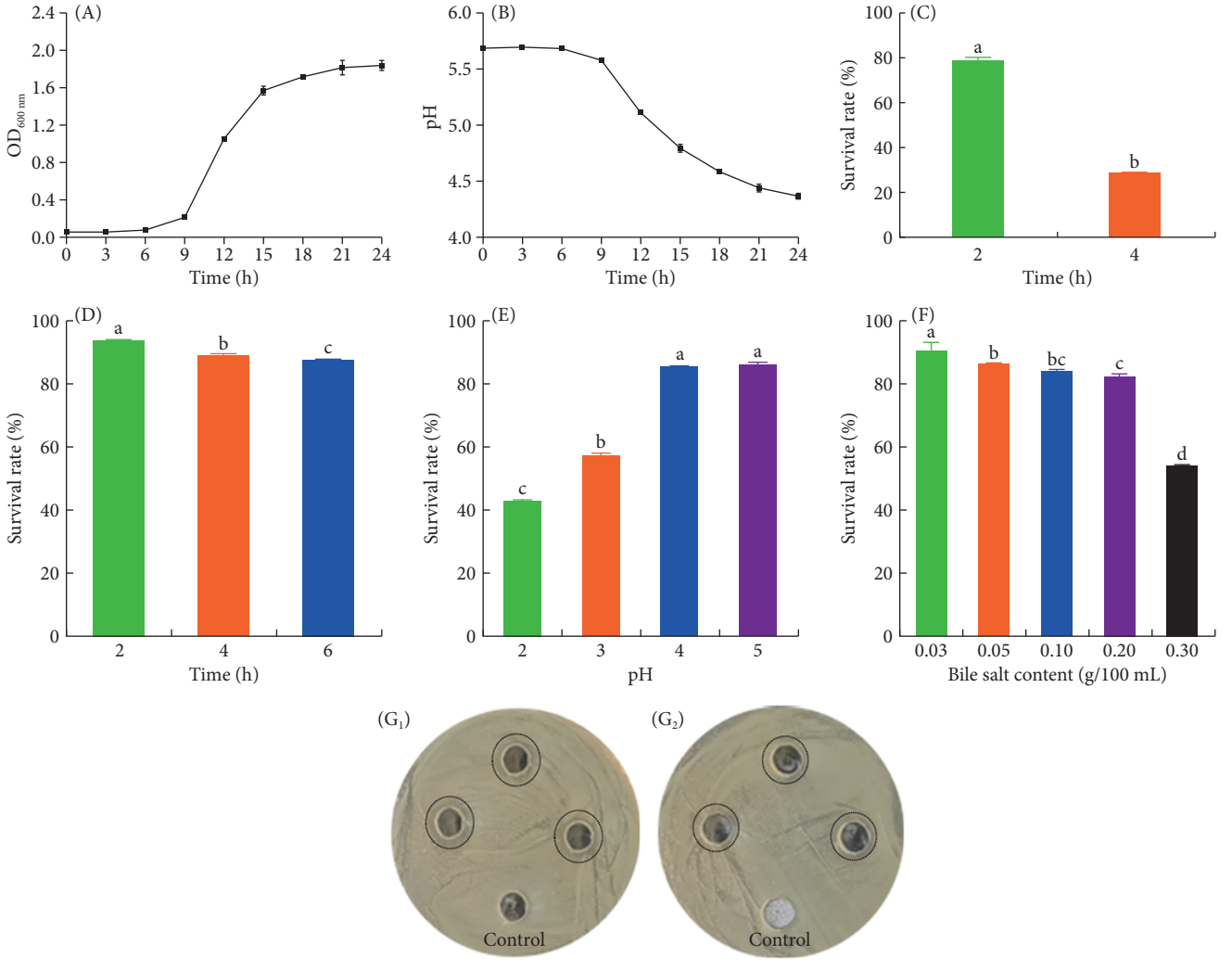

Figure 2 Growth characteristics, stress tolerance, and antibacterial activity of strain *L. sakei* CGMCC 23702. (A) Growth curve; (B) acid production capacity; (C) gastric fluid tolerance; (D) intestinal fluid tolerance; (E) acid tolerance; (F) bile salt tolerance; and antibacterial activity against (G_1) *E. coli* and (G_2) *S. aureus*. Bars labeled with different lowercase letters indicate significant differences among groups ($P < 0.05$).

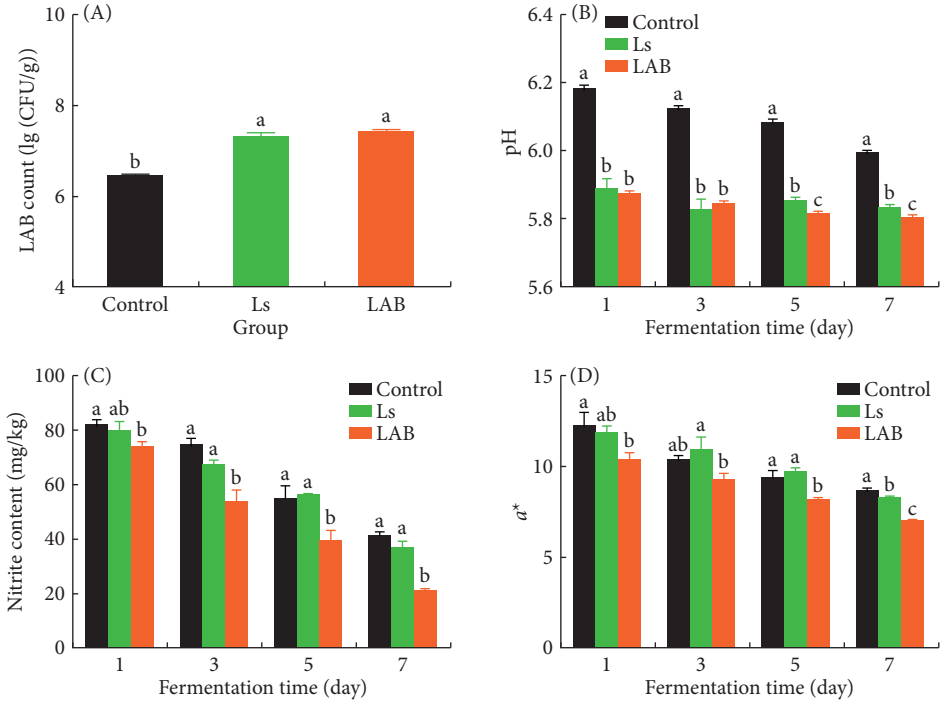

Figure 3 (A) LAB count, (B) pH, (C) nitrite content, and (D) *a** of fermented sausages prepared with natural fermentation, a single strain of *L. sakei*, and mixed LAB starter cultures during fermentation. Bars labeled with different lowercase letters indicate significant differences among groups ($P < 0.05$).

The hardness, elasticity, chewiness, and adhesiveness of the fermented sausages of each group in Table 1 show that the LAB group had excellent texture, especially the hardness was better at (4 298.00 ± 13.12) g. The antioxidant activity of the fermented duck sausages was lower in all groups (Figures 4A and B), while higher POV (Figure 4C) represented a higher degree of lipid oxidation. Overall, although there was little difference in DPPH radical scavenging activity, LAB presented better hydroxyl radical scavenging rate and POV compared to Ls.

3.3 Fluorescence spectral analysis of astaxanthin-wall material interaction

The results of fluorescence spectra showed that the strongest fluorescence emission peak appeared around 338 nm, and the fluorescence intensity decreased gradually with increasing astaxanthin concentration (0–100 μmol/L), while the shape of the emission peak remained unchanged, which indicated that the astaxanthin molecules could be inserted into the hydrophobic cavity of the wall material (Figure 5).

Table 1 Texture profile characteristics of fermented sausages prepared with natural fermentation, a single strain of *L. sakei*, and mixed LAB starter cultures.

Group	Hardness (g)	Elasticity (mm)	Adhesiveness (g)	Chewiness (g)
Control	3 618.00 ± 3.14^{c}	19.22 ± 0.02^{a}	816.00 ± 5.15^{b}	159.10 ± 0.52^{b}
Ls	3 759.00 ± 10.75^{b}	13.60 ± 1.01^{b}	968.00 ± 10.53^{a}	259.80 ± 0.44^{a}
LAB	4 298.00 ± 13.12^{a}	19.86 ± 0.12^{a}	652.00 ± 3.11^{c}	146.90 ± 0.55^{c}

Note: Values within a column marked with different lowercase superscript letters indicate statistically significant differences ($P < 0.05$).

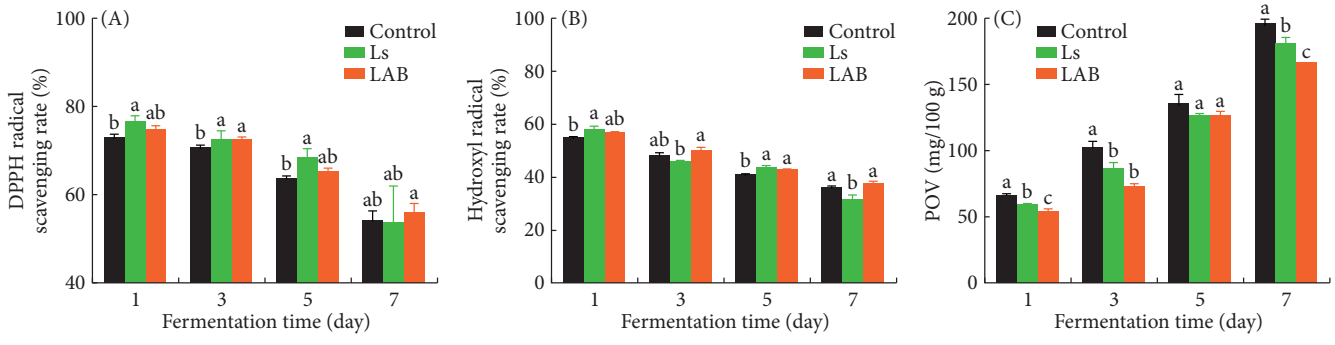

Figure 4 (A) DPPH radical scavenging rate, (B) hydroxyl radical scavenging rate, and (C) POV in fermented sausages prepared with natural fermentation, a single strain of *L. sakei*, and mixed LAB starter cultures during fermentation. Bars labeled with different lowercase letters indicate significant differences among groups ($P < 0.05$).

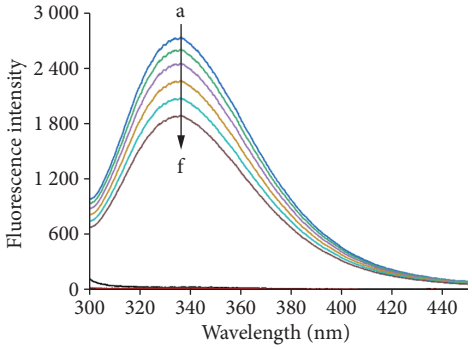

Figure 5 Effect of astaxanthin on the endogenous fluorescence of MFGM-WPI. a→f indicates astaxanthin concentrations of 0, 20, 40, 60, 80 and 100 μmol/L. The red line indicates the fluorescence spectrum of 20 μmol/L astaxanthin solution, the black line indicates the fluorescence spectrum of 0.5 mg/mL GA solution.

3.4 Microbiological, physico-chemical, and sensory features of fermented sausage with astaxanthin-rich nanoparticle compound LAB

Microbiological analysis showed that the LAB count in groups FA and NA was (6.50 ± 0.01) and (6.51 ± 0.01) (lg (CFU/g)), respectively, and the total number of LAB in groups FAL and NAL reached (7.94 ± 0.01) and (10.02 ±0.01) (lg (CFU/g)), respectively. The NAL was significantly higher than the FNL group ($P < 0.05$) (Figure 6A). As shown in Figure 6B, the pH of each sample progressively dropped until it reached a minimum after 7 days. Specifically, the NAL group had the lowest pH value of 5.810 ± 0.007 among the samples inoculated with LAB, with a lower pH than the non-inoculated group ($P < 0.05$). The nitrite content of the sausages steadily declined with the change in fermentation duration, with the NAL group having the lowest content of nitrite on day 7 of fermentation at (16.20 ± 1.41) mg/kg (Figure 6C). As the fermentation period rose, the a^* of all the sausages gradually increased, but the degree of change varied among the different groups. The FA and FAL groups showed a more rapid increase in reddening values, while the NA and NAL groups showed a more stable increase in reddening values (Figure 6D).

We investigated the effect of nano-astaxanthin and free astaxanthin on the texture of the sausages using chewiness, elasticity, adhesiveness, and hardness as indicators (Table 2). The NAL group had the highest hardness value of (4 524.00 ± 11.78) g; the FAL group followed with a hardness of (4 433.00 ± 9.87) g; Moreover, the hardness of both the NAL and FAL groups was significantly higher than that of the FA ((3 878.00 ± 13.78) g) and NA ((4 172.00 ± 12.71) g) groups ($P < 0.05$).

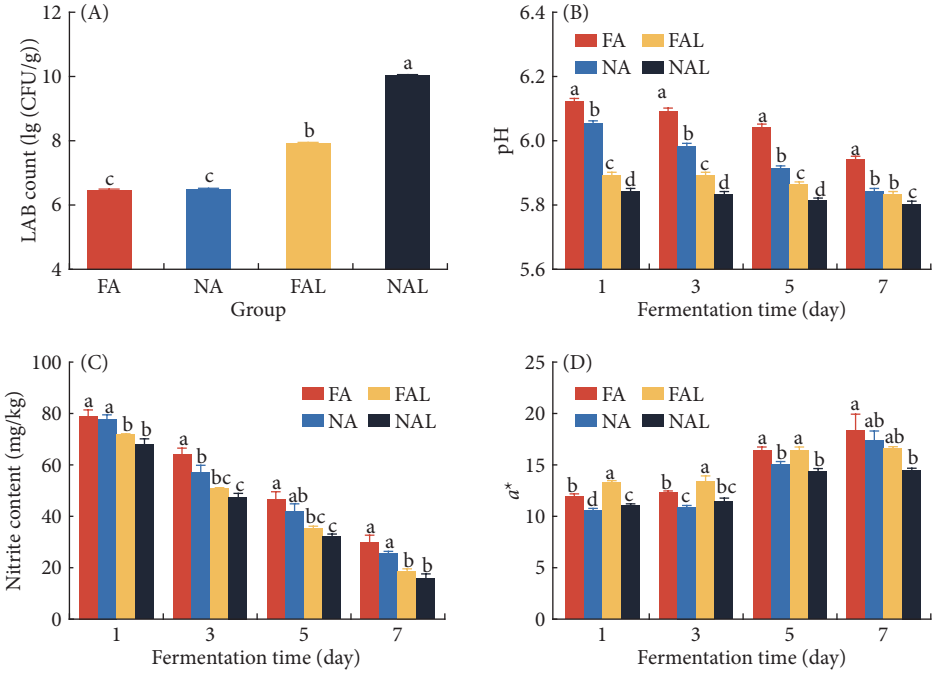

Figure 6 (A) LAB count, (B) pH, (C) nitrite content, and (D) a^* of fermented sausages supplemented with free astaxanthin, astaxanthin nanoparticles, free astaxanthin combined with LAB, and astaxanthin-loaded nanoparticles combined with LAB during fermentation. Bars labeled with different lowercase letters indicate significant differences among groups ($P < 0.05$).

Table 2 Texture profile characteristics of fermented sausages supplemented with free astaxanthin, astaxanthin nanoparticles, free astaxanthin combined with LAB, and astaxanthin-loaded nanoparticles combined with LAB.

Group	Hardness (g)	Elasticity (mm)	Adhesiveness (g)	Chewiness (g)
FA	3 878.00 ± 13.78^{d}	19.44 ± 0.01^{a}	756.00 ± 4.80^{a}	158.90 ± 2.14^{a}
NA	4 172.00 ± 12.71^{c}	19.57 ± 0.15^{a}	650.00 ± 11.10^{b}	158.50 ± 2.34^{a}
FAL	4 433.00 ± 9.87^{b}	19.76 ± 0.01^{a}	607.00 ± 7.70^{b}	152.80 ± 1.12^{a}
NAL	4 524.00 ± 11.78^{a}	19.79 ± 0.01^{a}	558.00 ± 9.56^{c}	147.10 ± 0.23^{a}

Note: Values within a column marked with different lowercase superscript letters indicate statistically significant differences ($P < 0.05$).

3.5 Astaxanthin retention and antioxidant properties of fermented sausage with astaxanthin-rich nanoparticle compound LAB

Due to the unstable nature of astaxanthin itself, astaxanthin retention rate decreases with increasing storage time (Figure 7A). The astaxanthin retention rate on the 7th day of fermentation was higher than 74% in both the NA and NAL groups, suggesting that the wall material had a superior encapsulating effect on astaxanthin, with the NAL group exhibiting a retention rate of up to (76.05 ± 0.21)%. As can be shown from Figures 7B and C, the fermented sausages' antioxidant activity increased when astaxanthin was added. On the 7th day of fermentation, the DPPH radical scavenging capacity was (75.65 ± 0.07)% (FA), (80.25 ± 0.07)% (NA), (77.25 ± 0.07)% (FAL), and (83.05 ± 0.21)% (NAL). Except for the NAL group ((51.25 ± 0.63)%), the other groups' hydroxyl radical scavenging rates ranged from 46% to 50%. As the fermentation time increased, the POV values of all groups showed an increasing trend (Figure 7D). Notably, the POV of the NAL group was always significantly lower than those of the other three groups on days 3, 5, and 7 of fermentation.

The NAL group showed a high number of live bacteria to achieve better acidity, and low nitrite content while ensuring the stability of the a^*, a high antioxidant activity to effectively prevent fat oxidation, and to ensure the flavor, color, and safety of the fermented duck sausages.

4 Discussion

LAB is the earliest bacterium applied as a fermentation agent for fermented meat products. Astaxanthin-loaded nanoparticles not only enhance bioactivity but also have an attractive micro-red color. Both LAB and astaxanthin nanoparticles are inextricably linked to the preparation of new fermented sausages. Our group has successively identified a combination of LAB effective in reducing nitrite and combined it with astaxanthin nanoparticles to produce fermented sausages with low nitrite content, better color, and antioxidant capacity. This study revealed that nitrite concentration was significantly reduced in fermented sausages with different starter cultures, and while *L. sakei* (screened for optimal nitrite-reducing ability due to its heme-dependent/independent nitrite reductases[6]) effectively depletes nitrite and improves microbiological quality[26]. Compound LAB fermentation outperformed single *L. sakei* fermentation—attributed to synergistic metabolites and pathway modulation from co-cultured strains[27]. Our compound LAB (six probiotic strains) exerts complementary roles, with clear correlations between strain functions and our experimental findings: 1) for faster pH decline: unlike *L. sakei* alone (9-h acid-production lag phase), *L. bulgaricus* and *L. plantarum* in the composite LAB achieved acid-production complementarity, rapidly lowering system pH to inhibit miscellaneous bacteria. *L. casei* and *L. acidophilus* further stabilized the acidification environment[28], laying the foundation for efficient fermentation. 2) For more thorough nitrite degradation: *L. sakei* (dominant strain, 92.16% degradation rate) is the core of nitrite conversion[6], while *L. casei* and *L. acidophilus* secrete auxiliary enzymes to form a complementary enzyme system. The rapid pH drop (promoted by *L. bulgaricus* and *L. plantarum*) further activates nitrite reductase, leading to significantly lower nitrite content in the LAB group. 3) For superior texture and quality: *L. bulgaricus*'s neutral/acid proteases decompose duck protein into free amino acids[29], optimizing the gel network; *L. plantarum* produces conjugated linoleic acid[30] to improve fat distribution and chewiness; *L. helveticus* enhances flavor compounds[28]. These synergies result in the LAB group's higher hardness ((4 298 ± 13.12) g) and denser texture, comprehensively improving sausage quality beyond single-strain fermentation.

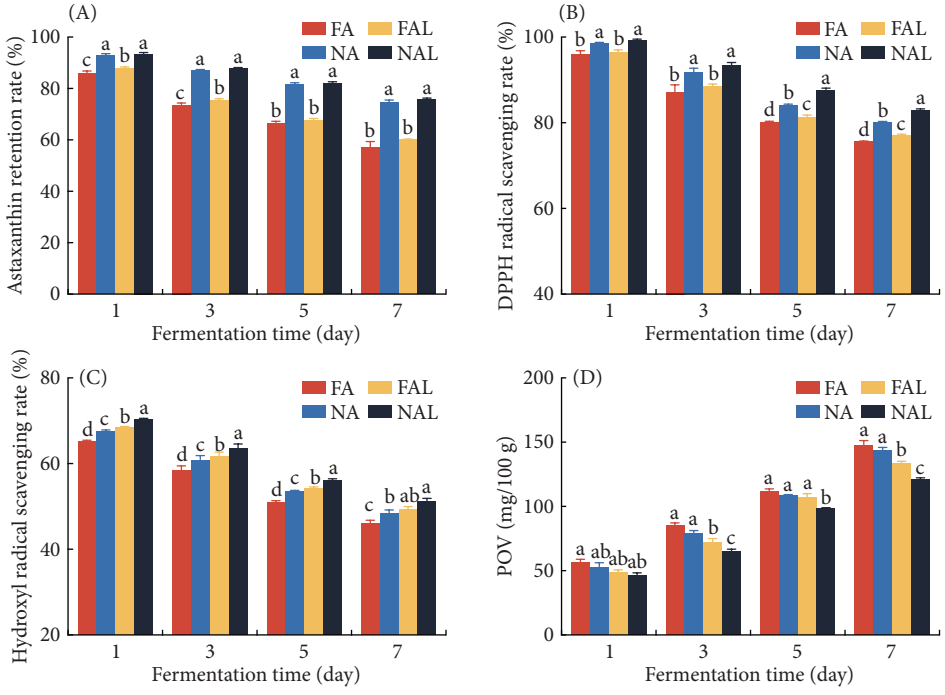

Figure 7 (A) Astaxanthin retention rate, (B) DPPH radical scavenging rate, (C) hydroxyl radical scavenging rate, and (D) POV of fermented sausages supplemented with free astaxanthin, astaxanthin nanoparticles, free astaxanthin combined with LAB, and astaxanthin-loaded nanoparticles combined with LAB during fermentation. Bars labeled with different lowercase letters indicate significant differences among groups ($P < 0.05$).

To overcome the limits of free astaxanthin in food applications, which were subject to very low water solubility, poor bioaccessibility, and low bioavailability[31], we designed MFGM-GA-astaxanthin nanoparticles employing the compound coalescence technique. This technique involved the coalescence of two or more water-soluble polymer compounds following a change in pH value, triggered by electrostatic interactions between oppositely charged entities[32]. In a binary mixture of a protein and polysaccharide, increasing the amount of polysaccharide molecules contributes to increasing the negative charges in the final complexes[33]. Taking into account polysaccharide molecules reported by Sepeidnameh et al.[34], GA was able to develop the outer layer in double-layer emulsions. When compared with protein molecules, polysaccharide molecules are highly capable of providing tightly packed interfacial films with higher thickness and can better entrap astaxanthin molecules in microcapsules. Similarly, a previous study[35] found prevention of lycopene degradation when encapsulated by complex coacervation in a gelatinegum arabic matrix. In addition, this compound coalescence technique, which provided versatility in formulation, such as MFGM and GA used in the experiment, enriches the functionality of the product.

Our research showed that *Lactobacillus* degrades the nitrite content and that the reduction of nitrite affects the color of the sausage. Therefore, we added astaxanthin-rich nanoparticles as a nitrite substitute to fermented sausages and studied the changes in color and antioxidant capacity of the sausages. We found reports of significant lipid oxidation and DPPH radical scavenging activity of astaxanthin in meat products[36]. Astaxanthin has been reported to have a desirable effect on antioxidant activity and the color of sausages, and it can be used as a multifunctional additive in sausages[37]. Evidence from this and similar studies suggested that the integration of astaxanthin into sausage formulations had a beneficial effect in terms of improving the quality of sausages[38]. Our results showed that the retention of astaxanthin in fermented sausages prepared with astaxanthin nanoparticles was significantly higher than that of free astaxanthin. This was in agreement with the report that astaxanthin nanoparticles exhibited greater stability and significantly improved antioxidant capacity compared to free astaxanthin[39]. It has also been reported that the obtained astaxanthin nanoparticles exhibited higher stability, were not affected by ultraviolet light, metal-induced oxidation, and thermal degradation, and showed high bioaccessibility of astaxanthin in simulated digestion experiments[40]. According to previous studies, the effect of astaxanthin on sausage color was determined by 11 conjugated double bonds (absorption maximum: 492 nm in dimethyl sulfoxide, 477 nm in acetone, and 477 nm in methanol) and astaxanthin could bind to proteins to further affect the color characteristics of different species[41]. Consequently, we investigated the effects of astaxanthin-loaded nanoparticles on a range of antioxidant properties and color indicators in sausages, further demonstrating the positive effects in sausages.

In addition to being rich in astaxanthin nanoparticles, the sausages were rich in MFGM, which was a complex biological membrane surrounding the fat globules in raw milk composed of polar lipids and specific MFGM proteins[42]. Because MFGM proteins possessed amphiphilic properties, they cannot only serve as natural emulsifying agents but also were considered good emulsifiers[43]. MFGM proteins have attracted wide attention owing to their various nutritional functions that have been reported, and among these functions, the most important properties involving effects on health are antibacterial, antiviral, and antiadhesive properties[44]. In addition, the differences among sausages made from free astaxanthin and astaxanthin-rich nanoparticles can be due to MFGM, which can promote the growth and metabolism of *Lactobacillus* during the fermentation process as a prebiotic[21]. Zhang et al.[45] reported that MFGM-derived phospholipids and glycoproteins could serve as dual-functional nutrients for *Lactobacillus rhamnosus*, not only providing carbon sources to enhance bacterial proliferation (1.2-fold higher viable count than the control group) but also regulating the expression of bacterial adhesion-related genes to improve their colonization ability in the meat matrix. Another study by Li et al.[46] further confirmed that MFGM-enriched whey protein significantly increased the viable count of *L. plantarum* in fermented products by 0.8 (lg (CFU/g)), and enhanced the bacteria's tolerance to acid and bile salts through modulating the integrity of bacterial cell membranes. GA, as an amphiphilic polysaccharide, was a weak polyelectrolyte carrying carboxyl groups, which can cross-link with positively charged particles through electrostatic interactions, and was often used as a food emulsifier and stabiliser due to its high solubility and low viscosity, which made it easy to bond with macromolecules[47]. However, more *in vivo* experiments related to antioxidant function remain to be explored, which is a subject of future research. *In vivo* experiments represent a critical future research priority: animal models should be constructed to clarify the *in vivo* antioxidant mechanism of astaxanthin nanoparticles, the metabolic fate of residual nitrite, and the bioavailability of encapsulated astaxanthin. Further exploration of the synergistic regulatory effects of composite LAB and astaxanthin nanoparticles on the host's intestinal flora and immune function will provide robust theoretical support for the industrialization of functional fermented meat products. In short, MFGM-GA-astaxanthin nanoparticles in this study are crucial in the meat industry, providing effective antioxidant preservation and offering a sustainable coloring option. This innovative additive supports a more sustainable and health-conscious approach to meat production, promising significant contributions to the industry's future.

5 Conclusion

In conclusion, we extracted a superior quality isolate *L. sakei* from salted fish and concurrently combined it with *L. helveticus*, *L. casei*, *L. bulgaricus*, *L. acidophilus*, and *L. plantarum* to serve as a starter for fermented sausages. In addition, MFGM-GA-astaxanthin nanoparticles were successfully constructed by the compound coalescence technique to add to this fermented sausage. The astaxanthin nanoparticles and LAB group were reddish in color, low in nitrites, rich in astaxanthin, with the strongest DPPH radical and hydroxyl radical scavenging activities, highest firmness, and best retention rate of astaxanthin. Thus, astaxanthin nanoparticles are a promising new natural additive with high application potential in probiotic fermented low-nitrite meat products.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported by the Jiangsu Provincial Frontier Technology Research and Development Program (BF2025322), the National Natural Science Foundation of China (32372355), the

Major Research Projects in Basic Sciences (Natural Sciences) of Higher Education Institutions in Jiangsu Province (25KJA550002), the National Key Laboratory for Hazard Factors and Risk Prevention and Control in Agricultural Product Quality and Safety Co-established by Provincial and Ministerial Authorities (2021DG700024-KF202424), the Research Institute of Nanjing Drum Tower Hospital (211420B52604), and the Forestry Science and Technology Innovation and Popularization Project in Jiangsu Province, China (LYKJ[2022]12).

Author contributions

Ping Ye: Analyzed the data and wrote the manuscript. Xiaozhi Wang, Xiaoyu Xu, Yage Wang, and Haibo Luo: Performed sample processing and experiments. Mingxuan Tao, Chen Liu, and Zi'ang Li: Preparation the fermented sausages. Xiaoqun Zeng, Zhen Wu, and Weimei Kong: Construction the astaxanthin nanoparticles. Daodong Pan and Yuxing Guo: Designed and performed the experiments, and supervised the manuscript. All authors read and approved the final manuscript.

References

[1] S. N. Kang, D. Yim, Effects of individual and combined doses of added nitrite and nitrate on the quality of dry-fermented sausage, Anim. Biosci. 38(6) (2025) 1272–1278. https://doi.org/10.5713/ab.24.0561.

[2] E. Dalzini, D. Merigo, A. Caproli, et al., Inactivation of *Listeria monocytogenes* and *Salmonella* spp. in Milano-type salami made with alternative formulations to the use of synthetic nitrates/nitrites, Microorganisms 10(3) (2022) 562. https://doi.org/10.3390/microorganisms10030562.

[3] J. Dalziel, D. Urwin, G. Band, et al., Rebound methaemoglobinaemia secondary to intentional sodium nitrite ingestion, Anaesth. Rep. 12(2) (2024) e12320. https://doi.org/10.1002/anr3.12320.

[4] L. Huang, X. Q. Zeng, Z. Sun, et al., Production of a safe cured meat with low residual nitrite using nitrite substitutes, Meat Sci. 162 (2020) 108027. https://doi.org/10.1016/j.meatsci.2019.108027.

[5] Q. Fan, C. R. Xia, X. Q. Zeng, et al., Effect and potential mechanism of nitrite reductase B on nitrite degradation by *Limosilactobacillus fermentum* RC4, Curr. Res. Food Sci. 8 (2024) 100749. https://doi.org/10.1016/j.crfs.2024.100749.

[6] J. W. Yuan, X. Q. Zeng, P. Zhang, et al., Nitrite reductases of lactic acid bacteria: regulation of enzyme synthesis and activity, and different applications, Food Biosci. 59 (2024) 103833. https://doi.org/10.1016/j.fbio.2024.103833.

[7] C. U. Mussagy, P. G. P. Silva, C. F. Amantino, et al., Production of natural astaxanthin by *Phaffia rhodozyma* and its potential application in textile dyeing, Biochem. Eng. J. 187 (2022) 108658. https://doi.org/10.1016/j.bej.2022.108658.

[8] S. Fakhri, F. Abbaszadeh, L. Dargahi, et al., Astaxanthin: a mechanistic review on its biological activities and health benefits, Pharmacol. Res. 136 (2018) 1–20. https://doi.org/10.1016/j.phrs.2018.08.012.

[9] Q. X. Zhou, L. Yang, J. Xu, et al., Evaluation of the physicochemical stability and digestibility of microencapsulated esterified astaxanthins using *in vitro* and *in vivo* models, Food Chem. 260 (2018) 73–81. https://doi.org/10.1016/j.foodchem.2018.03.046.

[10] Y. Gao, S. H. Yuan, Y. Z. Chen, et al., The improvement effect of astaxanthin-loaded emulsions on obesity is better than that of astaxanthin in the oil phase, Food Funct. 13(6) (2022) 3720–3731. https://doi.org/10.1039/d1fo03185f.

[11] Z. W. Liu, Y. X. Zhou, L. H. Wang, et al., Multi-spectroscopies and molecular docking insights into the interaction mechanism and antioxidant activity of astaxanthin and β-lactoglobulin nanodispersions, Food Hydrocoll. 117 (2021) 106739. https://doi.org/10.1016/j.foodhyd.2021.106739.

[12] N. Srihera, Y. Li, T. T. Zhang, et al., Preparation and characterization of astaxanthin-loaded liposomes stabilized by sea cucumber sulfated sterols instead of cholesterol, J. Oleo. Sci. 71(3) (2022) 401–410. https://doi.org/10.5650/jos.ess21233.

[13] E. Ngew, R. Kollipara, T. Bessissow, et al., Nanoencapsulation enhanced the performance of β-carotene for ameliorating inflammation in patient-derived organoids, Nanomedicine 20(7) (2025) 663–675. https://doi.org/10.1080/17435889.2025.2465247.

[14] R. R. Ambati, S. M. Phang, S. Ravi, et al., Astaxanthin: sources, extraction, stability, biological activities and its commercial applications: a review, Mar. Drugs 12(1) (2014) 128–152. https://doi.org/10.3390/md12010128.

[15] S. Chintong, W. Phatvej, U. Rerk-Am, et al., *In vitro* antioxidant, antityrosinase, and cytotoxic activities of astaxanthin from shrimp waste, Antioxidants 8(5) (2019) 128. https://doi.org/10.3390/antiox8050128.

[16] J. B. Bezelgues, F. Morgan, G. Palomo, et al., Short communication: milk fat globule membrane as a potential delivery system for liposoluble nutrients, J. Dairy Sci. 92(6) (2009) 2524–2528. https://doi.org/10.3168/jds.2008-1725.

[17] X. D. Zhang, X. Zhao, S. S. Tie, et al., Ultrasonic self-emulsification nanocarriers for cellular enhanced astaxanthin delivery, J. Agric. Food Chem. 69(9) (2021) 2719–2728. https://doi.org/10.1021/acs.jafc.0c05983.

[18] J. R. Cheng, S. W. Shen, H. G. Yang, et al., Improved physicochemical stability and bioaccessibility of astaxanthin-loaded oil-in-water emulsions by a casein-caffeic acid-glucose ternary conjugate, Food Res. Int. 163 (2023) 112153. https://doi.org/10.1016/j.foodres.2022.112153.

[19] K. Lee, A. Kim, K. B. Hong, et al., Preparation and characterization of a polar milk lipid-enriched component from whey powder, Food Sci. Anim. Resour. 40(2) (2020) 209–220. https://doi.org/10.5851/kosfa.2020.e5.

[20] M. Z. Liu, M. Liu, S. R. Yang, et al., Fermented milk of cheese-derived *Lactobacillus delbrueckii* subsp. *bulgaricus* displays potentials in alleviating alcohol-induced hepatic injury and gut dysbiosis in mice, Food Res Int. 157 (2022) 111283. https://doi.org/10.1016/j.foodres.2022.111283.

[21] M. F. Luo, Q. Y. Yuan, M. Z. Liu, et al., Astaxanthin nanoparticles ameliorate dextran sulfate sodium-induced colitis by alleviating oxidative stress, regulating intestinal flora, and protecting the intestinal barrier, Food Funct. 14(21) (2023) 9567–9579. https://doi.org/10.1039/d3fo03331g.

[22] X. Y. Liu, J. H. Ma, G. S. Fan, Microbiological safety and quality of fermented products, Foods 12(11) (2023) 2204. https://doi.org/10.3390/foods12112204.

[23] E. Morales, C. Burgos-Diaz, R. N. Zuniga, et al., Effect of interfacial ionic layers on the food-grade O/W emulsion physical stability and astaxanthin retention during spray-drying, Foods 10(2) (2021) 312. https://doi.org/10.3390/foods10020312.

[24] Y. W. Kong, M. Q. Feng, J. Sun, Novel antioxidant peptides in fermented pork sausage: purification, characterization, and cytoprotective functions on Caco-2 cells, Food Chem. 426 (2023) 136566. https://doi.org/10.1016/j.foodchem.2023.136566.

[25] M. M. Ozcan, D. Ipek, K. Ghafoor, et al., Physico-chemical and sensory properties of chips produced using different lupin (*Lupinus albus* L.) flour formulations and cooking methods, Int. J. Food Sci. Technol. 56(6) (2021) 2780–2788. https://doi.org/10.1111/ijfs.14913.

[26] G. Comi, D. Andyanto, M. Manzano, et al., *Lactococcus lactis* and *Lactobacillus sakei* as bio-protective culture to eliminate *Leuconostoc mesenteroides* spoilage and improve the shelf life and sensorial characteristics of commercial cooked bacon, Food Microbiol. 58 (2016) 16–22. https://doi.org/10.1016/j.fm.2016.03.001.

[27] J. J. Lee, Y. J. Choi, M. J. Lee, et al., Effects of combining two lactic acid bacteria as a starter culture on model kimchi fermentation, Food Res. Int. 136 (2020) 109591. https://doi.org/10.1016/j.foodres.2020.109591.

[28] J. Tian, X. Yang, K. Zhang, et al., Influence of *Lactobacillus helveticus* ZF22 and TR1-1-3 strains on the aromatic flavor of fermented sausages, Front. Nutr. 9 (2023) 1058109. https://doi.org/10.3389/fnut.2022.1058109.

[29] C. Q. Zhao, X. X. Zhao, Z. Y. Lu, et al., Production of fermented pork jerky using *Lactobacillus bulgaricus*, LWT-Food Sci. Technol. 72 (2016) 377–382. https://doi.org/10.1016/j.lwt.2016.04.060.

[30] C. O. Özer, B. Kılıç, Utilization of optimized processing conditions for high yield synthesis of conjugated linoleic acid by *L. plantarum* AB20-961 and *L. plantarum* DSM2601 in semi-dry fermented sausage, Meat Sci. 169 (2020) 108218. https://doi.org/10.1016/j.meatsci.2020.108218.

[31] F. N. Sorasitthiyanukarn, C. Muangnoi, P. Rojsitthisak, et al., Chitosan oligosaccharide/alginate nanoparticles as an effective carrier for astaxanthin with improving stability, *in vitro* oral bioaccessibility, and bioavailability, Food Hydrocoll. 124 (2022) 107246. https://doi.org/10.1016/j.foodhyd.2021.107246.

[32] P. Chowdhury, B. Saha, K. Bauri, et al., Hydrogen bonding-driven self-coacervation of nonionic homopolymers for stimuli-triggered therapeutic release, J. Am. Chem. Soc. 146(31) (2024) 21664–21676. https://doi.org/10.1021/jacs.4c05624.

[33] A. Bassijeh, S. Ansari, S. M. H. Hosseini, Astaxanthin encapsulation in multilayer emulsions stabilized by complex coacervates of whey protein isolate and Persian gum and its use as a natural colorant in a model beverage, Food Res. Int. 137 (2020) 109689. https://doi.org/10.1016/j.foodres.2020.109689.

[34] M. Sepeidnameh, S. M. H. Hosseini, M. Niakosari, et al., Physicochemical properties of fish oil in water multilayer emulsions prepared by a mixture of whey protein isolate and water-soluble fraction of Farsi gum, Int. J. Biol. Macromol. 118 (2018) 1639–1647. https://doi.org/10.1016/j.ijbiomac.2018.07.007.

[35] G. A. Rocha-Selmi, F. T. Bozza, M. Thomazini, et al., Microencapsulation of aspartame by double emulsion followed by complex coacervation to provide protection and prolong sweetness, Food Chem. 139(1) (2013) 72–78. https://doi.org/10.1016/j.foodchem.2013.01.114.

[36] E. Pogorzelska, J. Godziszewska, M. Brodowska, et al., Antioxidant potential of *Haematococcus pluvialis* extract rich in astaxanthin on colour and oxidative stability of raw ground pork meat during refrigerated storage, Meat Sci. 135 (2018) 54–61. https://doi.org/10.1016/j.meatsci.2017.09.002.

[37] J. K. Seo, R. Parvin, J. Park, et al., Utilization of astaxanthin as a synthetic antioxidant replacement for emulsified sausages, Antioxidants 10(3) (2021) 407. https://doi.org/10.3390/antiox10030407.

[38] I. Mohammadpourfard, A. Khanjari, A. Akhonzadeh Basti, et al., Evaluation of microbiological, chemical, and sensory properties of cooked probiotic sausages containing different concentrations of astaxanthin, thymol, and nitrite, Food Sci. Nutr. 9(1) (2021) 345–356. https://doi.org/10.1002/fsn3.2000.

[39] F. Yu, J. X. Chen, Z. Z. Wei, et al., Preparation of carrier-free astaxanthin nanoparticles with improved antioxidant capacity, Front Nutr. 9 (2022) 1022323. https://doi.org/10.3389/fnut.2022.1022323.

[40] F. Zanoni, M. Vakarelova, G. Zoccatelli, Development and characterization of astaxanthin-containing whey protein-based nanoparticles, Mar. Drugs 17(11) (2019) 627. https://doi.org/10.3390/md17110627.

[41] L. W. Sun, Y. Li, A. Q. Yang, et al., Astaxanthin: a comprehensive review of synthesis, biological activities and applications, Food Chem. 488 (2025) 144847. https://doi.org/10.1016/j.foodchem.2025.144847.

[42] S. H. He, H. S. Tang, H. X. Yi, et al., Properties of emulsions from milk fat globule membrane and its components, Int. J. Food Prop. 20(sup2) (2017) 1342–1353. https://doi.org/10.1080/10942912.2017.1343348.

[43] T. T. Q. Phan, T. T. Le, K. Dewettinck, Adsorption behavior at interface of oil-in-water emulsions prepared with mixtures of milk fat globule membrane proteins and polar lipids, Food Res. 4 (2020) 1744–1752. https://doi.org/10.26656/fr.2017.4(5).167.

[44] E. Saeland, M. A. de Jong, A. A. Nabatov, et al., MUC1 in human milk blocks transmission of human immunodeficiency virus from dendritic cells to T cells, Mol. Immunol. 46(11/12) (2009) 2309–2316. https://doi.org/10.1016/j.molimm.2009.03.025.

[45] L. L. Zhang, M. Chichlowski, G. Gross, et al., Milk fat globule membrane protects *Lactobacillus rhamnosus* GG from bile stress by regulating exopolysaccharide production and biofilm formation, J. Agric. Food Chem. 68(24) (2020) 6646–6655. https://doi.org/10.1021/acs.jafc.0c02267.

[46] P. Li, M. Liu, W. Lu, et al., Enhancing the survival of *Lactobacillus plantarum* via the rational design of whey protein isolate-high acyl gellan gum gelled water-in-oil emulsions, Colloids Surf. A Physicochem. Eng. Asp. 705 (2025) 135707. https://doi.org/10.1016/j.colsurfa.2024.135707.

[47] J. G. Ma, T. Z. Li, Q. Y. Wang, et al., Enhanced viability of probiotics encapsulated within synthetic/natural biopolymers by the addition of gum arabic via electrohydrodynamic processing, Food Chem. 413 (2023) 135680. https://doi.org/10.1016/j.foodchem.2023.135680.