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Impacts of exopolysaccharides producing probiotic bacteria on the physicochemical and sensory properties of fermented goat yoghurt under chilled storage

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Abstract: This study focused on the co-fermentation of goat milk with functional lactic acid bacteria (LAB) and traditional yoghurt starter culture. The fermentation process was optimised by single factor experiment and response surface methodology (RSM). The physicochemical and sensory properties of goat milk yoghurt were evaluated under chilled (4 °C) storage for 21 days. The optimised conditions were selected as the inoculum amount of *Lactobacillus paracasei* NM-8 (1.1×10^7 CFU·mL^{-1}; CFU – colony forming unit), sucrose addition (6.8%) and fermentation temperature (41 °C). During milk coagulation, the pH declined to be 4.45 and the viable LAB number arrived at 8.77 log CFU·mL^{-1}. The content of exopolysaccharides (EPS) increased to be 2.13 g·L^{-1}. These changes led to the better viscosity (941.33–792.33 cP) and higher water holding capacity (63.24–56.20%) of yoghurt fermented using *L. paracasei* NM-8 in storage, compared with those of yoghurt without *L. paracasei* NM-8. This study provided a theoretical basis for eliminating the whey precipitation and rough texture of goat milk yoghurt.

Keywords: goat milk yoghurt; lactic acid bacteria; optimisation; quality improvement; chilled storage

In recent years, goat milk has become a new favourite with great market prospects among consumers, due to its rich nutrients, lower allergenicity, higher digestibility, and better potential therapeutic benefits, compared to cow milk. Specifically, goat milk contained higher levels of conjugated linoleic acid (CLA), b-complex vitamins (riboflavin, thiamin, and B12), and minerals, such as calcium, phosphorus, and potassium (dos Santos et al. 2023). The average diameters of fat globules are approximately 2.76 μm in goat milk, which are less than those of 3.51 μm in the cow milk, contributing to a boosting digestibility of the former (Guo et al. 2022). As one of the alternative sources of animal proteins for people being allergic to cow milk, goat milk is also abundant in amino acids, bioactive peptides, fatty acids, lactose, oligosaccharides, and minerals (Ma et al. 2023). As previously reported, the meta-analysis and correlation of goat milk composition revealed

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that it might be of benefits to adults suffering from gastrointestinal disorders and ulcers (Akshit et al. 2024).

Yoghurt, a product derived from mammalian milk, has been an important component of human diet for centuries. As the demand for non-cow milk products increases, goat milk products, especially goat yoghurt, may be stand out for its advantages in the global consumer market. By using the proteins as substrate, goat milk is traditionally fermented with starter culture, namely *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* The type and content of proteins determine the yoghurt microstructure and the αs1-casein, one of the primary caseins in milk, plays a key role in the coagulation of yoghurt (Bijl et al. 2014). In this condition, there commonly exist the problems such as the whey precipitation and rough texture that impede the industrial production of goat milk yoghurt, due to the low casein content in goat milk.

As is well known, some lactic acid bacteria (LAB) are probiotics able to secrete the exopolysaccharides (EPS) as extracellular metabolites. The EPSs have been found possessing the antioxidant, antitumor, hypoglycemic, and intestinal flora regulation activities, and moreover, positively affecting the viscosity, hardness, and other organoleptic properties of foods (Lim et al. 2017). Ramchandran and Shah (2009) investigated the effects of EPS on the functional activities and rheological properties of low-fat yoghurt during storage. Their results showed that the EPS-involved yoghurt had the antihypertensive and antidiabetic properties and a stable and dense texture. Nevertheless, the studies concerning the optimisation of goat milk yoghurt manufacturing with co-inoculum of traditional starter culture and LAB that produces EPS, and simultaneously on the improvement of product quality, particularly the viscosity and water holding capacity (*WHC*) under chilled storage have not been reported.

This study evaluated the impacts of *Lactobacillus paracasei* NM-8, previously selected from traditional and spontaneous fermented foods with a great ability to produce EPS, on the physicochemical and sensory properties of goat yoghurt during storage. Our purpose was to explore the ultimate manufacturing process for the amelioration of goat yoghurt's quality, and thus provide the original strategy and fundamental theory for the development and industrialisation of fermented goat milk products using 'green approaches' without adding additives.

MATERIAL AND METHODS

Material. Goat milk powder was provided by Nanjing Haoming Dairy Co., Ltd. (China). A starter culture containing *Lactobacillus bulgaricus* (LB) and *Streptococcus thermophilus* (ST) was purchased from Weikang Probiotics Co., Ltd. (China). Man Rogosa Sharpe (MRS) broth was from Beijing Aoboxing Biotech Co., Ltd. (China). *L. paracasei* NM-8 was isolated from traditional fermented foods collected in Xinjiang province, China and was preserved in the China General Microbiological Culture Collection Center (CGMCC, China).

Preparation of goat milk yoghurt. According to the substance composition, the goat milk powder of the same batch was used to formulate 14% reconstituted milk (*w*/*v*) with warmed distilled water and was stirred until completely dissolved (Al-Wraikat et al. 2024). The reconstituted milk was then preheated to reach a temperature of 50–60 °C before the addition of sucrose and subsequent sterilisation at 108 °C for 15 min. The starter culture was inoculated at a certain dosage, accompanied with the inoculum of *L. paracasei* NM-8. After fermentation, the goat milk yoghurt was collected and allowed for preservation at 4 °C for 24 h and stored at 4 °C for 21 days.

Optimisation of goat milk yoghurt manufacturing. The effects of inoculation proportions of LB : ST starter culture (1 : 1, 1 : 1.5, 1 : 3, and 1 : 9), inoculum amounts of *L. paracasei* NM-8 (0.5×0^7, 1×10^7, 1.5×10^7, 2×10^7, and 2.5×10^7 CFU·mL^{-1}; CFU – colony forming unit), additive ratios of sucrose (2, 4, 6, 8, and 10%) and fermentation temperatures (35, 37, 39, 41, and 43 °C), on the basic characteristics of yoghurt products were investigated, respectively.

Based on the results of single-factor experiment, the inoculum amounts of *L. paracasei* NM-8 (0.5×10^7, 1×10^7, and 1.5×10^7 CFU·mL^{-1}), sucrose concentrations (4, 6, and 8%), and fermentation temperatures (39, 41, and 43 °C) were taken as the major influencing factors [see Electronic Supplementary Material (ESM), Table S1]. As shown in Table S2 in the ESM, the Box-Behnken design (BBD) was designed by using the Design-Expert software (version 13) to optimise the production process of goat milk yoghurt (Wu et al. 2015). The viscosity and water holding capacity (*WHC*) were set as the response indices.

Determination of exopolysaccharides content. The exopolysaccharides (EPS) content was determined as described by Wang et al. (2022) using the phenol-sulfuric acid method. Briefly, the cell-free supernatant of fermented goat milk was obtained via 4 500 rpm (revolutions per minute) centrifugation at 4 °C for 15 min. Thereafter, trichloroacetic acid (TCA, 80%) was added with a concentration of 4% (*v*/*v*), prior to the storage overnight at 4 °C. After the centrifuga-

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tion at 4 500 rpm for 15 min, the precipitate was collected and redissolved in distilled water for absorbance measurement at 590 nm. Glucose was used as standard to obtain a regression equation ($y = 0.0071x + 0.1591$, $R^2 = 0.9991$) for determining the sugar content. The EPS content was calculated as the difference between the sugar content of the fermented goat milk and that of the unfermented.

Determination of pH and titratable acidity. The pH value of yoghurt was determined using a pH meter and the acidity value was measured in accordance with reports by Wang et al. (2023a) with minor modifications. Briefly, approximately 10 g of yoghurt was weighed and mixed with 20 mL sterile water and 2 mL phenolphthalein indicator. Then, the mixture was titrated with 0.1 mol·L^{-1} sodium hydroxide solution. The titratable acidity was calculated as follows:

$$X = \frac{C_1 \times \left(V_1 - V_0\right) \times K \times 100}{m} \tag{1}$$

where: X – acidity (%); C_1 – molar concentration of NaOH solution (mol·L^{-1}); V_1 – volume of NaOH solution consumed during (mL); V_0 – volume of NaOH solution consumed in the control (mL); m – actual mass of yoghurt (g); K – conversion factor, i.e. the number of grams of lactic acid equivalent to the consumption of 1 mmol NaOH (0.09).

Determination of viable bacterial number. The number of viable LAB was determined by the plate count method using MRS agar (Wu et al. 2022). The yoghurt was treated using a gradient dilution with 0.85% sterile saline, and then 1 mL of diluent solution was taken into a sterile petri dish before incubation at 37 °C for 48 h. The confirmed colonies were counted and expressed as log CFU·mL^{-1}.

Determination of viscosity. The viscosity of yoghurt was measured using a DV-1+Pro Digital Viscosity meter with rotor type 3 (Jinghai, China) at 100 rpm, and the unit was expressed as cP.

Determination of water holding capacity. The water holding capacity (*WHC*) was determined as described by Bai et al. (2020) with slight modifications. The yoghurt was weighed and centrifuged at 4 500 rpm for 15 min at 4 °C, and then the sediment was also weighed. The *WHC* was calculated following the equation below:

$$WHC\ (\%) = \frac{W_1}{W_0} \times 100 \tag{2}$$

where: W_1 – sediment of yoghurt after centrifugation (g); W_0 – mass of yoghurt (g).

Sensory evaluation. The yoghurt manufactured with only traditional starter culture was set as the control group, while those co-inoculated with *L. paracasei* NM-8 and starter culture LB : ST were considered as the treatment group. The apparent colour, texture, taste, aftertaste, and overall acceptability of yoghurt were evaluated by 10 panelists, and the sensory evaluation criteria was listed in Table 1.

Table 1. Sensory evaluation criteria

Scoring item (score)	Standards
Colour (20)	15–20: uniform colour, glossy surface
	10–14: the colour is basically uniform, and the colour is dark
	0–9: the colour is uneven and grayish white
Texture (20)	15–20: fine texture, moderate viscosity, no whey precipitation
	10–14: the tissue is fine and there is a small amount of whey precipitation
	0–9: coarse texture, more whey precipitation
Taste (20)	15–20: the smell of mutton is faint and accompanied by a slight smell of frankincense
	10–14: the smell of goat is strong
	0–9: the smell of goat is very strong
Aftertaste (20)	15–20: delicate and smooth taste, sweet and tart
	10–14: average mouthfeel with slight graininess
	0–9: bad taste, grainy
Acceptability (20)	15–20: ideal and easy to accept
	10–14: feeling average, acceptable
	0–9: unacceptable

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Statistical analysis. All tests were carried out in triplicate and the results were expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to determine the significant differences between means ($P < 0.05$) using SPSS Statistics (version 26.0). Design-Expert (version 13) and Origin (version 2021) were applied for plotting.

RESULTS AND DISCUSSION

Exopolysaccharides producing ability of lactic acid bacteria

In our preliminary studies, a series of lactic acid bacteria strains were inoculated in goat milk for fermentation at 37 °C for 48 h, with the yield of exopolysaccharides ranging from 11.43 ± 1.66 to 200.16 ± 2.66 mg·L^{-1} (Figure 1). The content of EPS produced by *L. paracasei* NM-8 reached the highest at 200.16 ± 2.66 mg·L^{-1}. Ma et al. (2020) isolated the strain *Lactobacillus fermentum* HNUB20 that could produce EPS with a content of 98.8 mg·L^{-1} from fermented seafood. Moreover, Zhang et al. (2021) reported EPS yields for 20 strains of *Lactobacillus paracasei* to vary between 0.111 g·L^{-1} and 0.609 g·L^{-1}. The discrepancy in EPS yield produced by LAB strains probably depended on the differences in culture media, fermentation environment and some endogenous factors.

Optimisation of goat milk yoghurt manufacturing by single-factor test

Inoculation proportion of LB:ST. LB and ST are starter cultures commonly used in the fermentation of dairy products. LB produces many acids and peptides to induce the growth of ST, while ST generates formic acid to stimulate the growth and metabolism of LB (Li et al. 2023). They cooperate with each other and have a synergistic effect to advance the milk acidification and the growth of beneficial microbes during fermentation. The inoculation proportion of LB:ST was optimised under the fixed inoculum amount of 5 × 10^{7} CFU·mL^{-1}. As shown in Table 2, the pH and acidity of yoghurt gradually increased and dropped with the increasing proportions, respectively. On the other hand, the viable bacterial count arrived at the range of 8.57 ± 0.07 and 8.93 ± 0.06 log CFU·mL^{-1} when the proportions increased from 1:1 to 1:9. The viscosity and *WHC* were of 772.20 ± 14.30 cP and 59.86 ± 0.72%, respectively for the goat milk yoghurt containing 1:3 LB:ST, significantly higher than those of others ($P < 0.05$). Consistently, Surono (2022) reported that the optimum ratio of LB to ST in the starter culture for cow milk fermentation was between 1:5 to 2:1.

Inoculum amount of Lactobacillus paracasei NM-8. The effects of inoculum amount of *L. paracasei* NM-8 on the characteristics of yoghurt was indicated in Table 2. As the concentration of *L. paracasei* NM-8

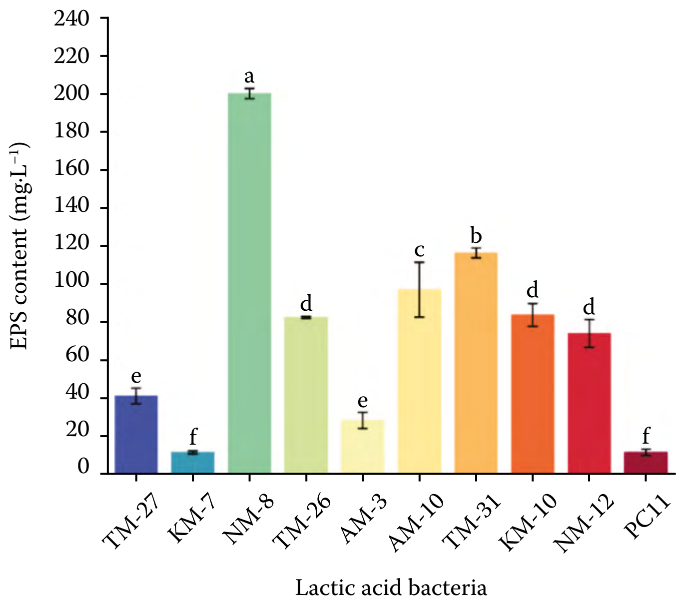

Figure 1. The contents of exopolysaccharides (EPS) produced by different lactic acid bacteria

a–f – mean values superscribed with different letters in the same column are significantly different ($P < 0.05$); TM-27, KM-10, KM-7, NM-12, TM-26 – abbreviations of *Lactobacillus paracasei* strains; AM-10, AM-3, PC11, TM-31, NM-8 – abbreviations of *L. rhanmosus* strains

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Table 2. Effects of different factors on characteristics of goat milk yoghurt

Factor		pH	Acidity (%)	Viscosity (cP)	*WHC* (%)	Viable count (log CFU·mL^{-1})
LB : ST	1 : 1	3.95 ± 0.01^{c}	1.40 ± 0.01^{a}	745.47 ± 16.81^{a}	58.09 ± 0.65^{b}	8.57 ± 0.07^{c}
	1 : 1.5	3.97 ± 0.02^{b}	1.35 ± 0.03^{b}	749.20 ± 21.61^{a}	58.30 ± 11.21^{b}	8.64 ± 0.11^{c}
	1 : 3	3.99 ± 0.01^{b}	1.35 ± 0.01^{b}	772.20 ± 14.30^{a}	59.86 ± 0.72^{a}	8.77 ± 0.05^{b}
	1 : 9	4.12 ± 0.01^{a}	1.24 ± 0.02^{c}	679.13 ± 3.60^{b}	56.25 ± 0.56^{c}	8.93 ± 0.06^{a}
Inoculum amount (CFU·mL^{-1})	0.5 × 10^{7}	4.17 ± 0.01^{a}	1.28 ± 0.01^{b}	859.00 ± 16.46^{b}	60.43 ± 0.34^{a}	8.80 ± 0.08^{b}
	1 × 10^{7}	4.08 ± 0.01^{c}	1.33 ± 0.02^{a}	911.67 ± 17.56^{a}	61.42 ± 1.41^{a}	8.99 ± 0.05^{a}
	1.5 × 10^{7}	4.14 ± 0.01^{b}	1.29 ± 0.02^{b}	873.53 ± 18.63^{b}	60.78 ± 0.73^{a}	8.94 ± 0.09^{a}
	2 × 10^{7}	4.12 ± 0.01^{b}	1.31 ± 0.01^{ab}	859.33 ± 10.07^{b}	60.46 ± 0.91^{a}	8.95 ± 0.04^{a}
	2.5 × 10^{7}	4.09 ± 0.01^{c}	1.32 ± 0.01^{a}	858.33 ± 9.09^{b}	60.29 ± 1.15^{a}	8.91 ± 0.02^{ab}
Sucrose addition (%)	2	4.17 ± 0.01^{d}	1.27 ± 0.01^{a}	655.67 ± 5.51^{e}	58.56 ± 3.89^{c}	8.73 ± 0.04^{c}
	4	4.20 ± 0.01^{c}	1.26 ± 0.00^{b}	699.27 ± 1.42^{d}	59.65 ± 0.62^{bc}	8.74 ± 0.02^{bc}
	6	4.19 ± 0.01^{cd}	1.26 ± 0.00^{b}	960.10 ± 10.00^{a}	64.40 ± 0.22^{a}	8.92 ± 0.04^{a}
	8	4.22 ± 0.01^{b}	1.25 ± 0.00^{b}	919.00 ± 21.00^{b}	62.74 ± 0.21^{ab}	8.87 ± 0.01^{a}
	10	4.25 ± 0.01^{a}	1.24 ± 0.00^{c}	767.67 ± 1.53^{c}	60.44 ± 0.50^{bc}	8.85 ± 0.03^{ab}
Temperature (°C)	35	4.60 ± 0.01^{a}	1.06 ± 0.01^{d}	528.67 ± 2.52^{c}	58.06 ± 2.19^{c}	9.08 ± 0.01^{a}
	37	4.16 ± 0.01^{d}	1.30 ± 0.01^{a}	864.67 ± 4.51^{b}	60.40 ± 0.77^{b}	9.00 ± 0.01^{c}
	39	4.18 ± 0.01^{d}	1.26 ± 0.01^{b}	959.20 ± 20.41^{a}	61.26 ± 0.65^{ab}	9.03 ± 0.00^{b}
	41	4.22 ± 0.01^{c}	1.25 ± 0.02^{bc}	971.67 ± 11.06^{a}	62.13 ± 0.54^{a}	8.91 ± 0.00^{e}
	43	4.29 ± 0.03^{b}	1.24 ± 0.00^{c}	978.81 ± 16.34^{a}	62.52 ± 1.06^{a}	8.97 ± 0.00^{d}

[a–e] Mean values superscribed with different letters in the same column are significantly different ($P < 0.05$); LB – *Lactobacillus bulgaricus*; ST – *Streptococcus thermophilus*; *WHC* – water holding capacity; CFU – colony forming unit

increased from 0.5 × 10^{7} to 2.5 × 10^{7} CFU·mL^{-1}, the acidity, viscosity and *WHC* of goat milk yoghurt fluctuated. The lowest pH was of 4.08 ± 0.01 and the highest acidity was of 1.33 ± 0.02% accordingly. The viscosity of yoghurt with an inoculum amount of *L. paracasei* NM-8 at 1 × 10^{7} CFU·mL^{-1} was of 911.67 ± 17.56 cP, which was significantly higher than those of others ($P < 0.05$). These results suggested that the *L. paracasei* NM-8 might cause insufficient acid production and inhibit other bacteria's growth. It was necessary to choose the amount of this strain suitable for the acidification and texture formation of goat milk yoghurt.

Additive ratio of sucrose. Sucrose addition in yoghurt products is crucial not only for the sweetness but also for promoting the growth of lactic acid bacteria. As indicated in Table 2, pH increased significantly with sucrose was added in a dose-dependent manner, and vice versa ($P < 0.05$). The addition ratios of 6–8% significantly increased the viable count from 8.73 ± 0.04 to 8.92 ± 0.04 log CFU·mL^{-1} ($P < 0.05$). The viscosity and *WHC* had similar variation tendencies to the viable count, reaching the highest of 960.10 ± 10.00 cP and 64.40 ± 0.22%, respectively at a sucrose concentration of 6% ($P < 0.05$). However, when the ratio of sucrose exceeded 8%, the LAB might proliferate rapidly and compete, leading to a lack of sufficient substrate for consumption.

Fermentation temperature. As shown in Table 2, the fermentation temperature obviously influenced the quality of goat milk yoghurt. There was a close relationship between fermentation temperature and milk coagulation, as lower temperature delayed the coagulation of milk proteins. Although the highest viable LAB count was determined to be 9.08 ± 0.01 CFU·mL^{-1} in yoghurt at 35 °C, in this circumstance exhibited the lowest acidity, viscosity, and *WHC*. With increasing temperature, the viscosity and *WHC* augmented significantly to be from 971.67 ± 11.06 cP to 978.81 ± 16.34 cP and from 62.13 ± 0.54% to 62.52 ± 1.06%, respectively at fermentation temperatures of 41–43 °C (Ps < 0.05; Ps was used when there was more than one index significantly different). Yang et al. (2021) conducted a multi-dimensional evaluation on the quality of yoghurt fermented at different temperatures, suggesting that the temperatures of 40–42 °C were favourable for enhancing the acidification and hardness of fermented goat milk.

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Optimisation of goat milk yoghurt manufacturing by response surface methodology

The response surface experiment was further used to optimise the manufacturing process and the analysis of variance was presented in Table 3. According to the values of R^2 and lack of fit for viscosity and *WHC*, the models were significant and had good precision and accuracy. The regression equations were obtained as follows:

$$\begin{aligned} Viscosity\ (\text{cP}) = {} & 931.42 + 35.18A + 12.06B + \\ & + 5.74C + 10.25AB - 2.00AC + \\ & - 4.72BC - 116.27A^2 - 41.70B^2 + \\ & - 49.65C^2 \end{aligned} \tag{3}$$

$$\begin{aligned} WHC\ (\%) = {} & 60.21 + 1.13A - 0.0508B - 0.1005C + \\ & + 0.7318AB + 2.03AC - 0.1284BC + \\ & - 0.8994A^2 + 0.3472B^2 - 0.4011C^2 \end{aligned} \tag{4}$$

where: A – inoculum amount; B – sucrose addition; C – temperature.

As indicated, the influence factor A had significant effects on the viscosity and *WHC* of yoghurt milk yoghurt, and moreover, the interactions of AB and AC had significant effects on the *WHC*, respectively (Ps < 0.05). The inoculum amount of *L. paracasei* NM-8 was therefore the most important factor that impacted on the texture of yoghurt.

The 3D response surfaces illustrated in Figure 2 provided a more intuitive view of the effects of various factors on the viscosity and *WHC* of yoghurt. The optimum manufacturing process could be determined as the inoculum amount of *L. paracasei* NM-8 of 1.1 × 10^7 CFU·mL^{-1}, sucrose addition of 6.8%, and fermentation temperature of 41 °C. In this condition, the actual values of viscosity and *WHC* were 921.67 ± 7.02 cP and 60.03 ± 0.90%, respectively and were close to the predicted values of the indices (938.7 cP and 60.40%).

Changes in pH and microbial growth

As seen from Figure 3, the pH of goat yoghurt dramatically declined in the early three hours, arriving at 5.27 ± 0.01 for yoghurt with only starter culture LB : ST and 5.03 ± 0.01 for that co-inoculated with *L. paracasei* NM-8 and LB : ST. Thereafter, the pH was gradually reduced to be about the isoelectric point 4.45–4.5 of goat milk protein, at which moment the gel structure of yoghurt formed along with the protein aggregation. It was indicated that the acid-producing capacity of lactic acid bacteria in existence of *L. paracasei* NM-8 was higher. Other study also reported that the yoghurt containing EPS had a higher acidity than that without EPS (Tiwari et al. 2021).

Moreover, Figure 3 demonstrated that the tendency of microbial growth in yoghurt with traditional starter culture was obviously different from that related

Table 3. Analysis of variance for response surface quadratic models

Source	Viscosity		*WHC*	
	F-value	*P*-value	*F*-value	*P*-value
Model	10.4000	0.0027	16.3600	0.0007
A	9.9700	0.0160	44.8500	0.0003
B	1.1700	0.3147	0.0908	0.7719
C	0.2654	0.6223	0.3553	0.5699
AB	0.4235	0.5360	9.4200	0.0181
AC	0.0161	0.9025	72.1200	< 0.0001
BC	0.0900	0.7729	0.2899	0.6070
A^2	57.3600	0.0001	14.9700	0.0061
B^2	7.3800	0.0299	2.2300	0.1790
C^2	10.4600	0.0144	2.9800	0.1281
Lack-of-fit	1.1700	0.4258	0.1629	0.9161
R^2	0.9304	–	0.9546	–
CV (%)	3.7800	–	0.7982	–
Adequate precision	8.6437	–	17.2471	–

A – inoculum amount; B – sucrose addition; C – temperature; *CV* – coefficient of variation; *WHC* – water holding capacity

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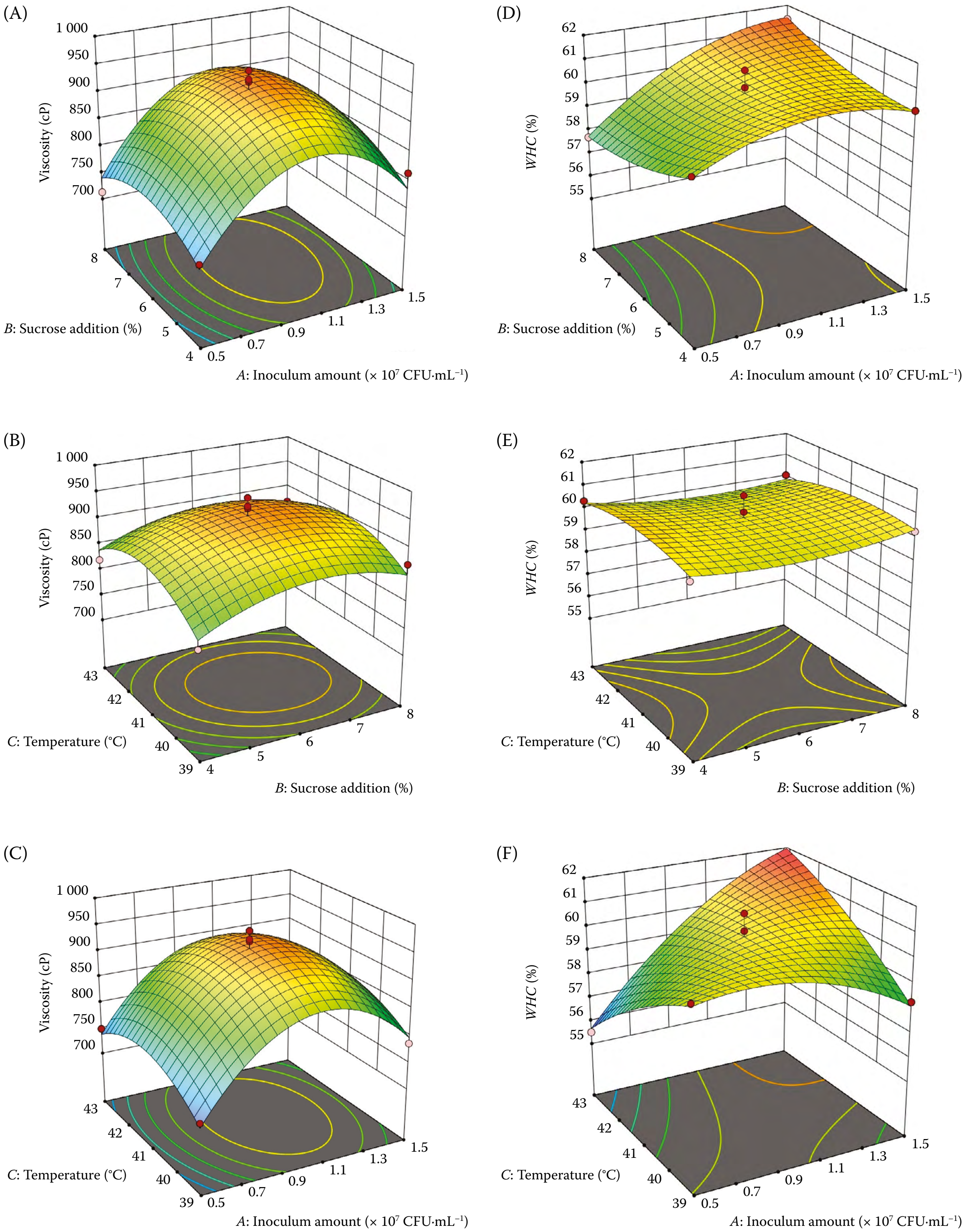

Figure 2. The response surface plots for goat milk fermentation with *Lactobacillus paracasei* NM-8: Effects of inoculum amount and sucrose addition on (A) viscosity and (D) *WHC*, (B) effects of sucrose addition and temperature on (B) viscosity and (E) *WHC*, and effects of inoculum amount and temperature on (C) viscosity and (F) *WHC*

CFU – colony forming unit; *WHC* – water holding capacity

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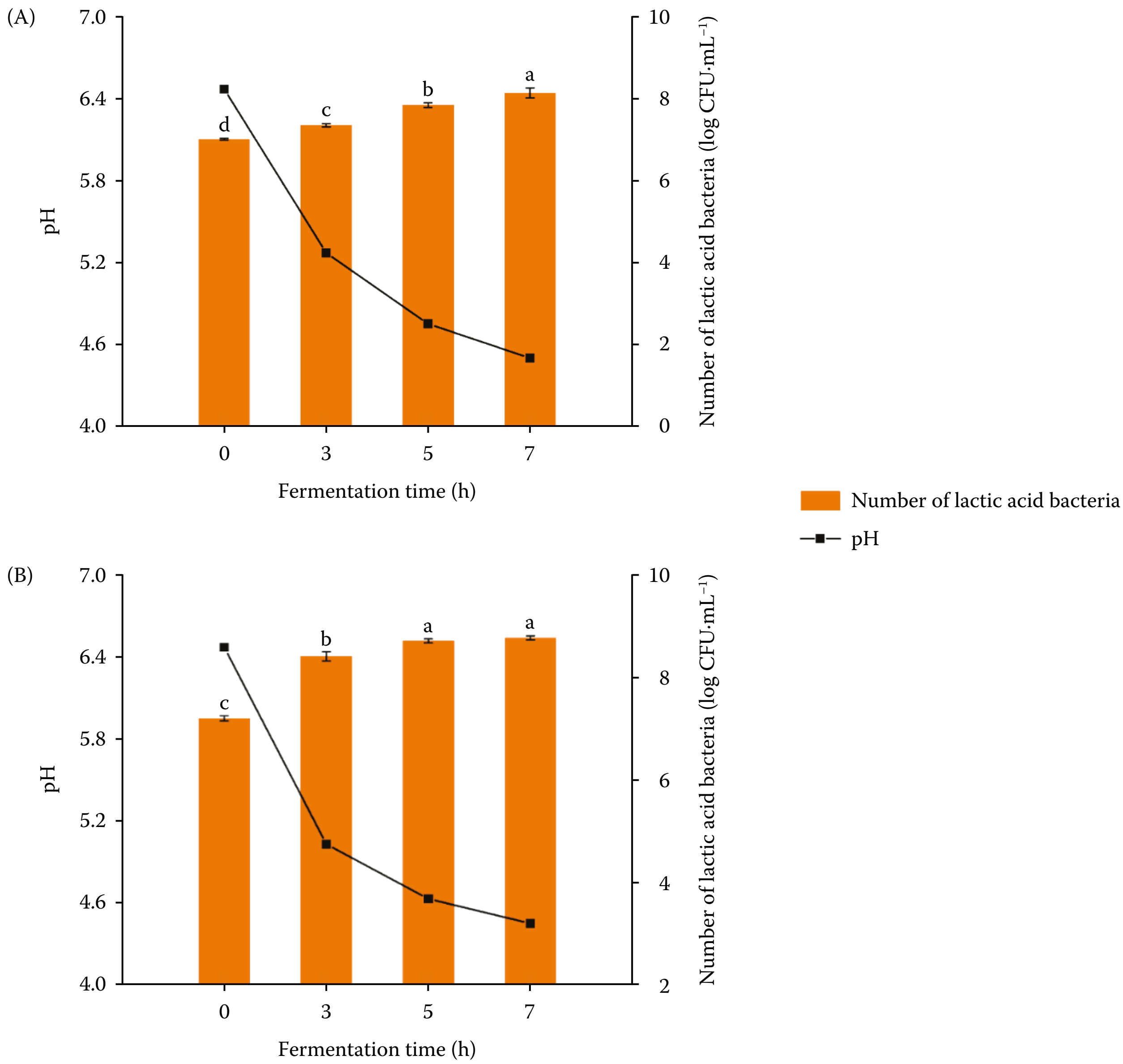

Figure 3. The changes in pH value and number of lactic acid bacteria during goat milk fermentation: (A) fermented goat milk without *Lactobacillus paracasei* NM-8 and (B) fermented goat milk with *L. paracasei* NM-8

a–d – mean values superscribed with different letters in the same column are significantly different ($P < 0.05$); CFU – colony forming unit

with *L. paracasei* NM-8. The control had the viable LAB numbers augmenting slowly from 7.01 ± 0.02 to 8.14 ± 0.12 log CFU·mL^{-1} ($P < 0.05$). The *L. paracasei* NM-8 accelerated the growth of LAB in goat milk, which increased largely from 7.2 ± 0.05 to 8.41 ± 0.09 log CFU·mL^{-1} during 0–3 h and changed slightly to be 8.77 ± 0.04 log CFU·mL^{-1} until the end of fermentation (Ps < 0.05). The International Dairy Federation (IDF) proposed that the active probiotic count of fermented milk products needed to be more than 10^{7} CFU·mL^{-1} (Li et al. 2022). As calculated, the strain *L. paracasei* NM-8 helped the fermented goat milk to have an increasing rate of microbial growth from 16.12% to be 21.81%.

Changes in exopolysaccharides content

The contents of exopolysaccharides in goat milk yoghurts during fermentation were shown in Figure 4. By using the LB : ST, the EPS content significantly increased to be 1.35 ± 0.15 g·L^{-1} in 5 h ($P < 0.05$) and remained stable during 5–7 h. The co-fermentation of goat milk with *L. paracasei* NM-8 and LB : ST led

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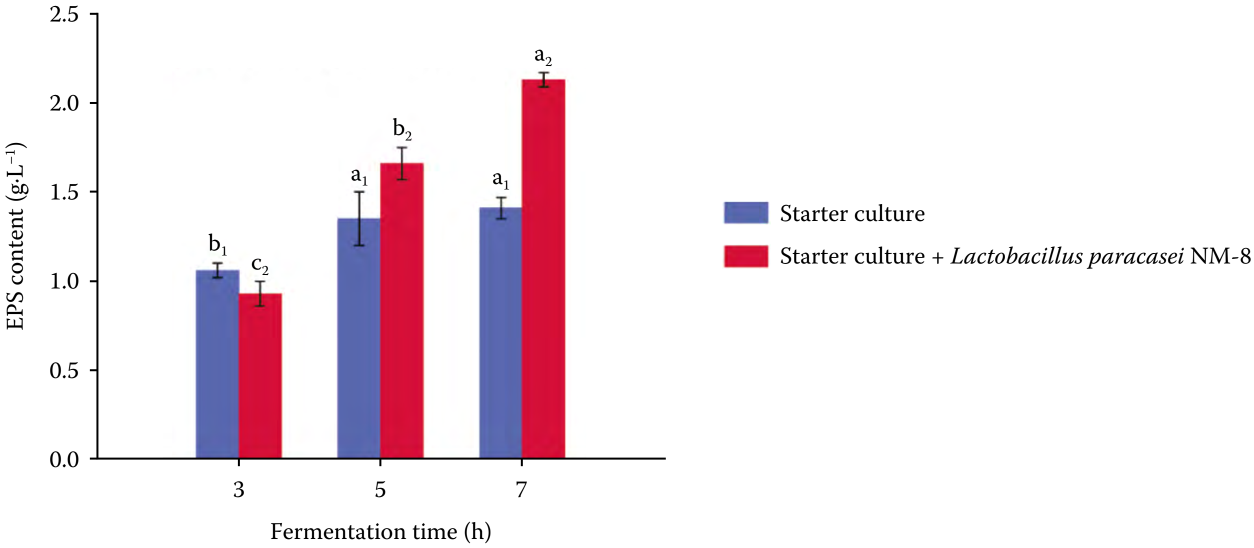

Figure 4. The changes in exopolysaccharides (EPS) content during goat milk fermentation

a_1–b_1 – significance for fermented goat milk without *Lactobacillus paracasei* NM-8 at the level of P < 0.05; a_2–c_2 – fermented goat milk with *L. paracasei* NM-8 at the level of P < 0.05

to a continuous increase in the EPS contents during fermentation, arriving at 2.13 ± 0.04 g·L^{-1} (P < 0.05). These results indicated that the existence of *L. paracasei* NM-8 ultimately accelerated the EPS production of lactic acid bacteria which could be used for goat milk yoghurt. It was worth noting that in the first three hours, the EPS content of the control yoghurt was higher than that of the *L. paracasei* NM-8 related yoghurt, probably due to the predominance of starter culture LB : ST in the early stage of fermentation.

Quality of goat milk yoghurt during storage

Physicochemical property. As presented in Figure 5A and 5B, under 21 days' storage at 4 °C, the pH of goat milk yoghurt inoculated with only starter culture LB : ST declined from 4.27 to 4.03, and slightly decreased from 4.17 to be 4.00 for the yoghurt with a mixture of *L. paracasei* NM-8 and LB : ST (Ps < 0.05). The acidity increased from 1.25% and 1.29% to be 1.35% and 1.36% at the 21st day for yoghurts without and with *L. paracasei* NM-8, respectively (Ps < 0.05). The changes in acidity showed an opposite tendency of the pH due to the accumulation of organic acids, and this phenomenon might be known as 'post-acidification' (Yue et al. 2022). Besides the 'post-acidification', the living LAB also contributed to the production of acids during storage. Notably, the *L. paracasei* NM-8 strain with EPS producing ability led to less increase of acidity in the period of 15–21 days. Consistently, Yang et al. (2023a) reported that the addition of *Potentilla anserine* polysaccharide (PAP) retarded the progress of post acidification in yak yoghurt during storage.

The changes in viscosity and *WHC* of different yoghurts under chilled storage were displayed in Figure 5C and 5D. As indicated, the viscosity varied from 903 ± 6.08 to 709.2 ± 1.71 cP for the yoghurt with starter culture LB : ST and from 941.33 ± 23.44 to 792.33 ± 10.69 cP for that co-fermented with both *L. paracasei* NM-8 and LB : ST (Ps < 0.05). The *WHC* dropped from 60.78 ± 0.66% and 63.24 ± 1.13% to 50.60 ± 0.78% and 56.20 ± 0.53% for yoghurts without and with *L. paracasei* NM-8, respectively (Ps < 0.05). The remarkable decline of indices reflected the texture destruction of yoghurt during storage (Wang et al. 2023b). By comparison, the control had a decrease of viscosity and *WHC* by 21.48% and 16.75%, respectively, more than those involved with *L. paracasei* NM-8 of 15.83% and 11.13%, respectively. The goat milk yoghurt manufactured with the traditional starter culture and *L. paracasei* NM-8 had improved texture of products and higher level of quality maintainance under chilled storage, which was probably explained by the ability of *L. paracasei* NM-8 to produce the EPS.

Sensory property. According to the sensory evaluation criteria, the sensory characteristics of yoghurt during storage were investigated and described as the radar plot shown in Figure 6A. The total scores of yoghurts without and with *L. paracasei* NM-8 were calculated as 69.9 and 79 on the first day, respec-

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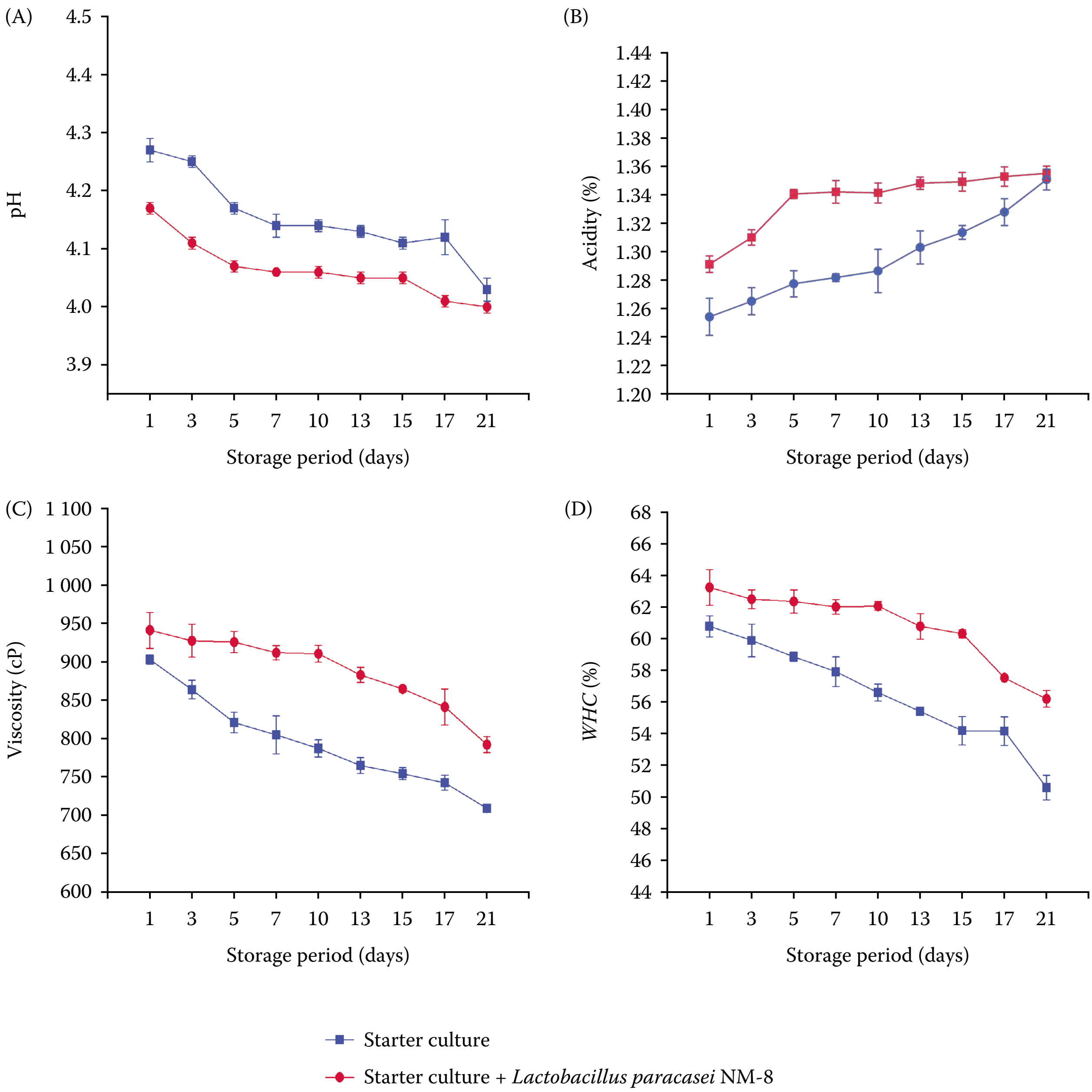

Figure 5. The physicochemical properties of fermented goat milk during 21 days chilled storage: (A) pH, (B) acidity, (C) viscosity, and (D) water holding capacity (*WHC*)

tively. After storage for 21 days at 4 °C, the *L. paracasei* NM-8 helped the fermented goat milk to have a higher total score of 60 than the control of 45.55. Detailly, as for the control, the scores of colour, acceptance, aftertaste, taste and texture dropped by 35.98, 36.09, 39.18, 31.25, and 33.07%, respectively, and these scores decreased only by 26.95, 24.53, 29.17, 22.07, and 15.33%, respectively for the co-inoculated yoghurt. It was indicated that the *L. paracasei* NM-8 played an important role in improving the sensory qualities of goat milk yoghurt before and after the storage. Çakmakçi et al. (2012) similarly reported the enhanced sensory quality and probiotic properties of yoghurt by adding *Bifidobacterium bifidum*. On the other hand, the *L. paracasei* NM-8 performed best on the maintenance of texture than of the other indices, which was agreed with our above findings. As shown in Figure 6B, the serious whey precipitation occurred in the yoghurt fermented in absence of *L. paracasei* NM-8 after storage.

Although the goat milk yoghurt is becoming increasingly popular, its development and industrial produc-

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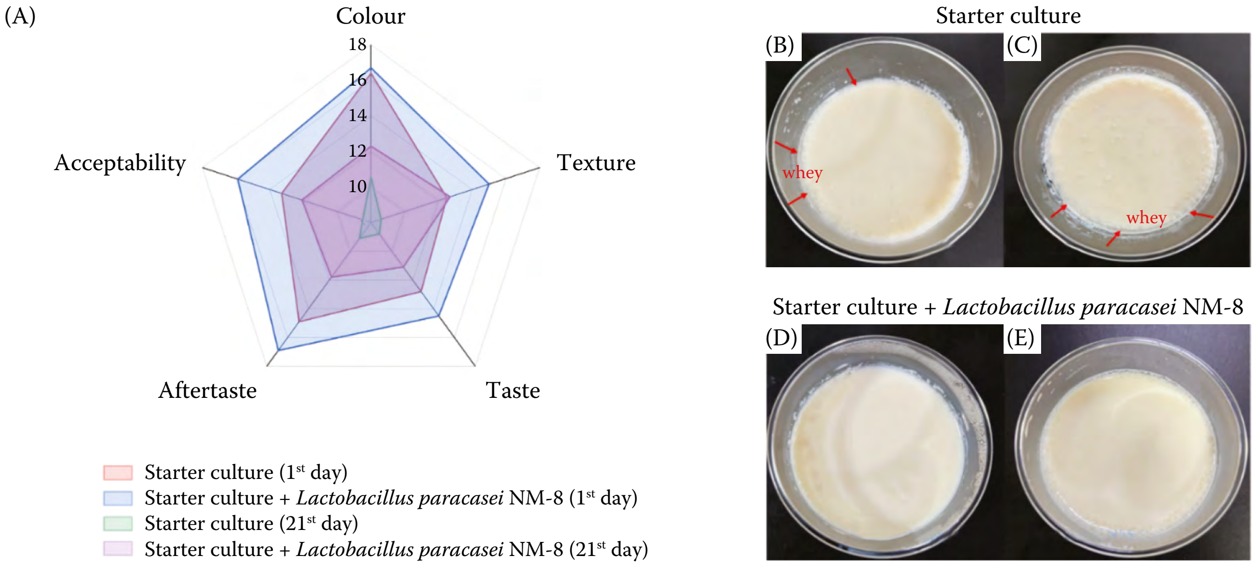

Figure 6. The sensory properties of fermented goat milk after 21 days chilled storage: (A) sensory evaluation radar chart; (B–E) appearance inspection, fermented goat milk (B–C) without *Lactobacillus paracasei* NM-8 after (B) 1st and (C) 21st days' storage, and (D–E) with *L. paracasei* NM-8 after (D) 1st and (E) 21st days' storage

tion still have some challenges, e.g. whey precipitation, rough texture, and sensory issues. As previously reported, De Santis et al. (2019) used both a starter culture and *Leuconostoc lactis* to enhance the yoghurt's sensory quality, and Yang et al. (2023b) also stated that the appropriate inoculum amount of *L. casei* effectively alleviated the undesirable flavour of fermented goat milk. Our results demonstrated that the co-inoculation with *L. paracasei* NM-8 and traditional starter culture could be a natural and safe strategy to improve the quality of goat yoghurt. Our findings could further provide a theoretical reference for the future development of goat milk products by using certain probiotic strains with special functionalities.

CONCLUSION

In this study, a lactic acid bacteria strain namely *L. paracasei* NM-8 that produces exopolysaccharides was selected and applied in the manufacturing of goat milk yoghurt. The fermentation process was optimised by response surface experiment and the effects of *L. paracasei* NM-8 on the physicochemical and sensory properties of yoghurt were also evaluated. The inoculation with *L. paracasei* NM-8 could be one of the natural and safe measures to attenuate the whey precipitation and improve the sensory property of yoghurt under chilled storage. Our findings helped provide a theoretical reference for the development of goat milk products via the use of functional probiotics.

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REFERENCES

Akshit F.N.U., Mao T., Kaushik R., Poswal V., Deshwal G.K. (2024): Global comprehensive review and meta-analysis of goat milk composition by location, publication year and lactation stage. Journal of Food Composition and Analysis, 127: 105973.

Al-Wraikat M., Abubaker M.A., Liu Y.L., Shen X.P., He Y., Li L.Q., Liu Y.F. (2024): Label-free quantitative proteomic analysis of functional changes of goat milk whey proteins subject to heat treatments of ultra-high-temperature and the common low-temperature. Food Chemistry, 10: 101691.

Bai M., Huang T., Guo S., Wang Y., Wang J., Kwok L.Y., Dan T., Zhang H., Bilige M. (2020): Probiotic *Lactobacillus casei* Zhang improved the properties of stirred yoghurt. Food Biosciences, 37: 100718.

Bijl E., de Vries R., van Valenberg H., Huppertz T., van Hooijdonk T. (2014): Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. International Dairy Journal, 34: 134–141.

Çakmakçi S., Çetin B., Turgut T., Gürses M., Erdogan A. (2012): Probiotic properties, sensory qualities, and storage stability of probiotic banana yoghurts. Turkish Journal of Veterinary & Animal Sciences, 36: 231–237.

De Santis D., Giacinti G., Chemello G., Frangipane M.T. (2019): Improvement of the sensory characteristics of goat milk yoghurt. Journal of Food Science, 84: 2289–2296.

dos Santos W.M., Guimarães Gomes A.C., de Caldas Nobre M.S., de Souza Pereira Á.M., dos Santos Pereira E.V., dos Santos K.M.O., Florentino E.R., Alonso Buriti F.C. (2023): Goat milk as a natural source of bioactive compounds and strategies to enhance the amount of these beneficial components. International Dairy Journal, 137: 105515.

Guo S., Chen M., Wu T., Liu K., Zhang H., Wang J. (2022): Probiotic *Bifidobacterium animalis* ssp. *lactis* Probio-M8 improves the properties and organic acid metabolism of fermented goat milk. Journal of Dairy Science, 105: 9426–9438.

Li H., Song W., Liu T., Xu S., Zhang S., Zhang Y., Liu D., Li H., Yu J. (2022): Developing novel synbiotic yoghurt with *Lacticaseibacillus paracasei* and lactitol: Investigation of the microbiology, textural and rheological properties. International Dairy Journal, 135: 105475.

Li Z.Q., Shao Y.C., Gong Y.X., Wu W.R., Cao L.R., Li B.X., Lu Y.Y. (2023): Effect of starter inoculation proportions on the attributes of a superior new variety *Osmanthus* glutinous rice-fermented milk beverages. International Journal of Food Science and Technology, 58: 4529–4542.

Lim J., Kale M., Kim D.H., Kim H.S., Chon J.W., Seo K.H., Lee H.G., Yokoyama W., Kim H. (2017): Antiobesity effect of exopolysaccharides isolated from kefir grains. Journal of Agricultural and Food Chemistry, 65: 10011–10019.

Ma C., Guo H., Chang H., Huang S., Jiang S., Huo D., Zhang J., Zhu X. (2020): The effects of exopolysaccharides and exopolysaccharide-producing *Lactobacillus* on the intestinal microbiome of zebrafish (*Danio rerio*). BMC Microbiology, 20: 300.

Ma J., Wang Q., Dou N., Li Y., Ma Y., Liu Y., Wu M., Wei X., Miao Y., Chen L., Xu D., Hou J., Jiang Z. (2023): Evaporative concentration and high-pressure homogenization for improving the quality attributes and functionality of goat milk yoghurt. LWT – Food Science & Technology, 184: 115016.

Ramchandran L., Shah N.P. (2009): Effect of exopolysaccharides and inulin on the proteolytic, angiotensin-I-converting enzyme- and α-glucosidase-inhibitory activities as well as on textural and rheological properties of low-fat yoghurt during refrigerated storage. Dairy Science & Technology, 89: 583–600.

Surono I.S. (2022): Fermented milks: Starter cultures. In: Encyclopedia of Dairy Sciences. 3rd Ed. New York, Academic Press: 477–482.

Tiwari S., Kavitake D., Devi P.B., Halady Shetty P. (2021): Bacterial exopolysaccharides for improvement of technological, functional and rheological properties of yoghurt. International Journal of Biological Macromolecules, 183: 1585–1595.

Wang X., Xu M., Xu D., Ma K., Zhang C., Wang G., Dong M., Li W. (2022): Structural and prebiotic activity analysis of the polysaccharide produced by *Lactobacillus helveticus* SNA12. Carbohydrate Polymers, 296: 119971.

Wang L., Wu T., Zhang Y., Yang K., He Y., Deng K., Liang C., Gu Y. (2023a): Comparative studies on the nutritional and physicochemical properties of yoghurts from cows', goats', and camels' milk powder. International Dairy Journal, 138: 105542.

Wang Y., Li D., Chitrakar B., Zhang X., Zhang N., Liu C., Li Y., Wang M., Tian H., Li C. (2023b): Copper inhibits postacidification of yoghurt and affects its flavor: A study based on the *Cop* operon. Journal of Dairy Science, 106: 897–911.

Wu H., Rui X., Li W., Chen X.H., Jiang M., Dong M.S. (2015): Mung bean (*Vigna radiata*) as probiotic food through fermentation with *Lactobacillus plantarum* B1-6. LWT – Food Science & Technology, 63: 445–451.

Wu H., Liu H.N., Ma A.M., Zhou J.Z., Xia X.D. (2022): Synergetic effects of *Lactobacillus plantarum* and *Rhizopus oryzae* on physicochemical, nutritional and antioxidant properties of whole-grain oats (*Avena sativa* L.) during solid-state fermentation. LWT – Food Science & Technology, 154: 112687.

Yang S., Yan D., Zou Y., Mu D., Li X., Shi H., Luo X., Yang M., Yue X., Wu R., Wu J. (2021): Fermentation temperature affects yoghurt quality: A metabolomics study. Food Biosciences, 42: 101104.

Yang J., Sun J., Yan J., Zhang X., Ma Y., Liu C., Du P., Li A. (2023a): Impact of *Potentilla anserine* polysaccharide on storage properties of probiotic yak yoghurt. International Dairy Journal, 141: 105585.

Yang Y., Zhang R., Zhang F., Wang B., Liu Y. (2023b): Storage stability of texture, organoleptic, and biological properties of goat milk yoghurt fermented with probiotic bacteria. Frontiers in Nutrition, 9: 1093654.

Yue Y., Wang S., Lv X., Wang C., Xu B., Ping L., Guo J., Li X., Evivie S.E., Liu F., Li B., Huo G. (2022): Analysis of the complete genome sequence of *Lactobacillus delbrueckii* ssp. *bulgaricus* with post-acidification capacity and its influence on yoghurt in storage. Journal of Dairy Science, 105: 1058–1071.

Zhang Y., Dai X., Jin H., Man C., Jiang Y. (2021): The effect of optimized carbon source on the synthesis and composition of exopolysaccharides produced by *Lactobacillus paracasei*. Journal of Dairy Science, 104: 4023–4032.

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