Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Evaluation of the Technological Characteristics of a *Lacticaseibacillus casei* ANNC 26 Strain Isolated from Traditional Khiki Cheese as a Co-Starter Culture

Behrooz Alizadeh Behbahani[1*], Parisa Ghasemi[2], Alireza Vasiee[3], Zeinab Mousavi[4]

1- Associate Professor, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

2- PhD student, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

3- Assistant Professor, Department of Food Safety and Quality Control, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran.

4- M.Sc. student, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

ARTICLE INFO

Article History:

Received: 2026/01/04
Review: 2026/02/01
Accepted: 2026/02/08

Keywords:

Traditional Khiki Cheese,

Exopolysaccharide,

Acidifying Ability,

Lacticaseibacillus casei.

DOI: 10.48311/fsct.2026.118719.83022

*Corresponding Author E-Mail:

B.alizadeh@asnrukh.ac.ir

ABSTRACT

The functional efficacy of starter cultures in industrial settings relies heavily on their resilience to stress. This study comprehensively evaluated the key technological properties of an indigenous *Lacticaseibacillus casei* ANNC 26 strain isolated from traditional Khiki cheese; thermal resistance, exopolysaccharide (EPS) production, acidifying ability, and enzymatic activities. The thermal resistance assessment showed the strain possessed significant stability against heat stress. The highest survival (57.42%) was recorded at 50 °C, and even at 80 °C, a 25.73% survival was observed, indicating its resilience under thermal conditions. The strain showed significant EPS synthesis, induced most strongly by sucrose and glucose, validating its potential as a multifunctional bio-additive for enhancing texture and water retention. With an acidifying activity (ΔpH24h=1.48), the strain was classified as a Moderate Acidifier, making it ideal for use as an Adjunct Culture in long-ripened cheeses requiring gradual pH reduction. Furthermore, it demonstrated high proteolytic activity (21.69 ± 0.09 mm halo diameter) and "good" autolytic activity. These high enzymatic activities confirm its potential to release flavor precursors and bioactive peptides. Collectively, these favorable characteristics confirm the indigenous *L. casei* ANNC 26 strain as a promising Adjunct Starter candidate for significantly enhancing the texture, flavor, and stability of Khiki cheese, thereby supporting its industrial development.

1-Introduction

Fermented foods have long been recognized across various global cultures as one of the oldest and most effective methods for preserving and enhancing the quality of food products. This product category has garnered significant attention in recent studies not only for its extended shelf-life but also for its notable sensory characteristics, superior nutritional value, and inherent health benefits [1]. Lactic Acid Bacteria (LAB), primarily belonging to the genera *Lactobacillus*, *Streptococcus*, and *Leuconostoc*, are considered the main and predominant microorganisms in fermentation processes. Their core activity is the production of lactic acid, which causes a rapid reduction in environmental pH, thereby dramatically improving the microbial safety of the food matrix [2,3]. This pH decrease helps inhibit the growth of both pathogenic and spoilage microorganisms, ultimately ensuring the safety of the final products. Furthermore, LAB play a key and undeniable role in numerous fermented products, such as yogurt, kefir, cheese, and other traditional foods, contributing significantly to flavor development, texture enhancement, and the fortification of nutritional value [3,4]. Many LAB strains also possess intrinsic probiotic properties, capable of conferring positive health effects on the host through mechanisms such as boosting the immune system, producing vitamins, and synthesizing beneficial metabolites [5,6]. These functional and health-promoting features have led to the targeted utilization of high-performance LAB strains becoming a major priority for the modern food industry in the development of Functional Foods and health-oriented products.

Khiki cheese (or brine-ripened cheese) represents a prominent example of traditional, widely consumed dairy products in the diets of many nomadic and rural areas, historically serving as an essential source of protein and nutrients. This type of cheese is typically stored in a highly concentrated salt solution (brine), and its ripening process occurs under saline and acidic conditions. These specific chemical and environmental characteristics create a highly hostile environment for the proliferation of most microorganisms [7]. However, the high concentration of salt and acidity during ripening, besides affecting the final texture and flavor, also increases the challenges associated with processing and quality control at an industrial scale [8]. Nevertheless, these harsh environmental conditions act as a natural selection mechanism, promoting the growth and persistence of specific LAB strains that possess unique survival characteristics in stressed environments [9]. These indigenous strains often demonstrate high tolerance to osmotic and acidic stresses and are thus considered valuable resources for use as starter cultures or industrial, resilient probiotics [10]. Recent studies have also clearly indicated that the microbial diversity and metabolic activity of these indigenous strains have a direct impact on the sensory quality, flavor development, and safety of the final Khiki cheese product [4,11]; these characteristics underscore the crucial importance of investigating and functionally evaluating indigenous LAB strains in traditional products like Khiki cheese.

Given that Khiki cheese is traditionally produced, often without the use of pure industrial starters, LAB strains isolated from these natural environments are expected to exhibit superior technological traits capable of addressing industrialization challenges. These features include high thermal resistance (thermotolerance) to ensure survival after initial milk treatment processes (such as mild pasteurization), rapid medium acidification capability, and appropriate proteolytic and autolytic activity for the fast development of desirable flavor and texture [12,13]. Furthermore, the ability of these strains to produce EPS can significantly contribute to improved water retention, increased consistency, and structural stability within the saline environment [14]. Such characteristics make these strains suitable candidates for the development of specialized indigenous starters and for enhancing the quality and shelf-life of Khiki cheese [11]. Consequently, the systematic evaluation of these key technological properties in LAB strains isolated from Khiki cheese is considered an essential step toward the industrialization of this traditional product while preserving its authenticity and nutritional value. Therefore, the present study aimed at a comprehensive evaluation of the technological properties of the indigenous *L. casei* ANNC 26 strain isolated from Khiki cheese, including its thermal resistance, EPS production capability, acidification rate, proteolytic activity, and autolytic activity; ultimately, this research

sought the successful application of this strain as an Adjunct Starter culture to enhance the texture quality, flavor, and stability of Khiki cheese, thereby promoting the industrial development of this traditional product.

2- Materials and Methods

2.1. Materials

In this study, the probiotic strain *L. casei* ANNC 26, isolated from Traditional Khiki Cheese, was utilized. This strain was maintained in a lyophilized state at the laboratory of Khuzestan University. All culture medium (MRS broth, MRS agar, and MRSC agar) were procured from the Quelab (Canada) brand.

2.2. Probiotic Strain Activation

For the activation of the *L. casei* ANNC 26 strain, 100 µL of the lyophilized culture was transferred to MRS broth and incubated anaerobically at 37 °C for 48 h. Following activation, the resulting culture was utilized for the evaluation of the strain's technological properties.

2.3. CFS Preparation

To prepare the Cell-Free Supernatant (CFS) from the *L. casei* ANNC 26 strain, 100 µL of the activated strain was first inoculated into MRS broth. The resulting culture was then incubated under anaerobic conditions at 37 °C for 24 h. Subsequently, the bacterial culture was centrifuged at 9,000 rpm for 10 min at 4 °C to separate the CFS. The collected supernatant was then passed through a 0.22 µm filter to ensure sterilization. Finally, the sterile filtrate was lyophilized (freeze-dried) for concentration and subsequent use in assays.

2.4. Thermal resistance

L. casei ANNC 26 cells were cultivated in MRS broth at 37 °C under static (anaerobic) culture conditions. To ensure physiological homogeneity, cells were harvested during the exponential growth phase (OD_{600}=0.6) by centrifugation (6,000 rpm for 10 min at 4 °C). The resulting cell pellets were washed twice with phosphate buffer before being resuspended in the same buffer for final dilution. The resulting suspension was then subjected to an acute heat stress treatment for 20 minutes in a controlled water bath at variable temperatures of 50 °C, 60 °C, 70 °C, and 80 °C. Following the heat treatment, samples were incubated anaerobically for 16 h at 37 °C for cellular recovery. Finally, cellular viability was determined and reported as the percentage of colony-forming ability (used as an indirect growth index) by measuring the optical density at 600 nm (OD600) using a spectrophotometer [15].

2.5. Exopolysaccharide production

EPS production by the *L. casei* ANNC 26 strain was evaluated based on the methodology described by Ogunremi et al. (2022), with minor modifications [16]. For this purpose, 10 µL of the actively grown culture (obtained after 24 h of incubation in MRS broth at 37 °C) was spot-inoculated onto the surface of various media. These media included MRSC agar (MRS medium supplemented with 0.25% L-cysteine) and MRSC agar further supplemented with 0.2% of different sugars, namely glucose, lactose, fructose, and sucrose. All samples were subsequently incubated under anaerobic conditions at 30 °C for 48 h. EPS production in the resulting colonies was then assessed visually; this evaluation was performed based on the observable characteristics of the slime mass, or ropiness, produced by the colonies.

2.6. Acidifying ability

The acidifying potential of the *L. casei* ANNC 26 strains was evaluated by examining pH changes in milk cultures [17]. Actively growing bacterial cultures were inoculated at a 1% ratio into 100 ml of sterile, reconstituted skim milk. The pH of the culture medium was measured using a pH meter. pH values were recorded at both the start of incubation (time zero) and after 6 and 24 h of storage at 30 °C. Finally, the extent of the pH drop (ΔpH) for the 6 h and 24 h intervals were calculated using the following formula (1):

$$\Delta pH = pH_0 - pH_{6,\,24}$$

2.7. Proteolysis activity

The proteolytic activity of the isolate under investigation was assessed by introducing slight modifications to the method of Saci et al. (2025) [18]. For this purpose, a well 6 mm in diameter was bored into the surface of MRS agar that had been reinforced with the addition of 10% (v/v) sterile skim milk. Subsequently, 70 µL of the CFS obtained from this strain was accurately transferred to the aforementioned well. Finally, following a 24 h incubation period at 30 °C, the diameter of the clear zone, which is the index of positive proteolytic activity, was measured and recorded.

2.8. Autolytic activity

The autolytic activity of the *L. casei* ANNC 26 strain was quantitatively evaluated using the methodology described by Davati et al. (2016), for the purpose of determining the rate of cellular viability decrease [19]. Following strain activation, cells from the overnight culture were collected by centrifugation (600 rpm, 10 min, 4 °C). The resulting cell pellet was washed twice with phosphate buffer to completely remove the culture medium. Subsequently, the purified pellet was resuspended in the same buffer, and its concentration was standardized to an OD600 of 1.0. Then, the cell suspension was subjected to a cold stress cycle that involved 24 h of exposure to both 4 °C and −20 °C; after exiting this cycle, the samples were incubated at 30 °C for 24 h to initiate the autolysis process. Finally, the autolysis rate was calculated based on the reduction in optical density at 600 nm after 24 h of incubation, using the following formula (2):

$$\text{Autolytic activity (\%)} = [(A_{initial} - A_{final})/A_{initial}] \times 100 \quad (2)$$

Were, $A_{initial}$ and A_{final} respectively denote the initial optical density of the cell suspension, which serves as a measure of the starting cell concentration, and the final optical density recorded following the designated incubation period.

2.9. Statistical analysis

Data were analyzed using One-Way Analysis of Variance (ANOVA) with SPSS statistical software (version 26) to assess the significance of observed differences. Duncan's multiple range test was used for mean comparisons. All statistical tests were conducted at a significance level of $p < 0.05$, and the experiment was performed in triplicate.

3-Results and Discussion

3.1. Thermal resistance

The adaptability and resilience to various stressors are considered the primary drivers of bacterial evolution, ensuring cell survival in unfavorable environments [20]. Nevertheless, the survival and operational efficacy of LAB in industrial processing environments are heavily dependent on their thermotolerance. The strain's thermal resistance exhibited a continuous and significant downward trend in response to increasing temperatures (Fig. 1). The highest level of stability was observed at 50 ºC with a mean survival rate of 57.42%, which was statistically superior to all other thermal conditions ($p < 0.05$). This decline in survival, which dropped to 25.73% at 80 ºC, clearly underscores the increased selective pressure resulting from industrial stresses [21]. High thermal stress primarily damages the cell through protein denaturation, although membranes, nucleic acids, and certain enzymes have also been identified as sensitive targets of heat injury. Furthermore, thermal stress causes a disruption of the transmembrane proton gradient, subsequently leading to the acidification of the cytoplasmic environment [22]. Therefore, the comprehensive assessment and quantification of LAB strains' thermal tolerance are not only essential for the targeted selection of industrial candidate strains but also serve as a fundamental tool for optimizing processing parameters and enhancing the stability and quality of the final biotechnological products. Previous studies have similarly reported the thermal resistance of LAB strains, emphasizing the variability among different strains and the critical importance of this trait against routine industrial heat treatments [23,24].

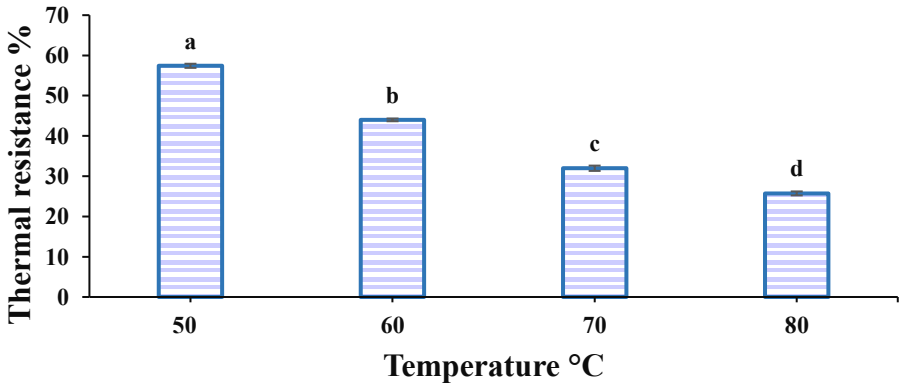

Fig. 1. Thermal resistance of *L. casei* ANNC 26 at various temperatures. Different letters indicate significant differences ($p < 0.05$).

3.2. Exopolysaccharide production

The assessment of the EPS synthesis capacity by microbial strains is conducted through screening protocols based on the cultivation of LAB in media enriched with various sugars, including glucose, fructose, sucrose, galactose, or lactose. The simplest and most customary criterion for initial EPS production detection is the examination of colony phenotypic characteristics. This approach is primarily founded upon distinguishing between colonies that exhibit either a slimy or a ropy morphology. The slimy phenotype is characterized by a mucilaginous (mucoid) colony texture, while the ropy phenotype is determined by the cells' ability to form long elastic strands when an inoculation loop is lifted from the colony surface or cell pellet [25]. In line with these methodologies, our observations in the current study confirmed the *L. casei* ANNC 26 strain's significant capacity for EPS production, which manifested as prominent mucoid halos surrounding the colonies. This phenotypic expression was directly influenced by the nature of the carbon source provided in the growth medium. The visual assessment of carbon sources indicated that sucrose and glucose were the strongest inducers of EPS production, eliciting the most pronounced mucoid morphology. In contrast, although the EPS production phenotype was also observed in media containing fructose and lactose, this expression was clearly less intense than under sucrose and glucose conditions, suggesting a lower efficacy of these sugars in promoting the intensity of extracellular polymer expression in the strain under investigation. These findings are consistently supported by previous research. For example, observations by Santal et al. (2019) and Bacosa et al. (2018) reported that the LAB strains examined in their studies also exhibited EPS-producing colonies with a mucoid appearance [26,27]. Furthermore, the study by Minari et al. (2024), which assessed the EPS production capability of the *Lacticaseibacillus casei* (Ke8) strain using glycerol, glucose, and molasses as carbon sources, demonstrated that glucose is one of the most effective growth substrates for EPS synthesis [28]. Similarly, the work by Cerning et al. (1994), which investigated the carbon source requirements of *L. casei* (CG11) in a minimal medium (comparing glucose, sucrose, lactose, galactose, maltose, and melibiose), confirmed that glucose was the most strikingly efficient source for stimulating EPS production, while lactose and galactose showed inferior performance [29]. These extensive results emphasize the high dependence of EPS production on the type of carbon substrate. Due to these synthetic capabilities, EPS produced by LAB plays a significant functional and technological role across various food industries. In bakery products and cereals, these biopolymers increase bread volume, retain moisture, improve texture, and prevent staling; they also aid in the formation of a structural network in gluten-free products [30,31]. Moreover, in fermented vegetable and plant products, EPS enhances the viscosity, mouthfeel, and physical stability of pureed products, such as carrot purée [32] In the meat industry, particularly in low-fat fermented sausages, EPS acts as a water-holding agent and texture improver, thereby reducing the need for chemical additives [33]. These wide-ranging applications firmly establish EPS as a versatile multifunctional bio-additive with high industrial potential across diverse food sectors.

3.3. Acidifying ability

Lactic acid production stands as a critical metric for assessing starter culture performance, as this acid not only provides the desirable fresh, tangy flavor in dairy products but also plays a pivotal role in curd tissue formation by rapidly reducing the medium's pH. The ability of *Lactobacillus* species to metabolize sugars and rapidly produce acid is directly correlated with enhancing desirable sensory attributes, ensuring the safety and durability of final products by inhibiting pathogen growth, and ultimately, validating the strains as effective starters. Thus, optimal acidifying activity is a fundamental prerequisite for the application of LAB in fermentation industries [34]. Based on recent studies, LAB strains are categorized into three primary groups according to the pH drop (ΔpH24h) observed after 24 hours of acid production: Weak Acidifiers (ΔpH24h≤1.11), Strong Acidifiers (ΔpH24h≥1.61), and Moderate Acidifiers whose ΔpH24h ranges from 1.12 to 1.60 [35]. Results from the evaluation of the *L. casei* ANNC 26 strain's acidifying activity (ΔpH24=1.48) indicate that this strain is classified as a Moderate Acidifier (Fig. 2). This level of pH reduction (within the 1.12 to 1.60 range) suggests that the *L. casei* ANNC 26 strain possesses an adequate sugar metabolism rate for lactic acid production; however, it is not

on par with Fast Starters that reduce the pH below 5.0 in under 6 h. This characteristic makes the *L. casei* strain highly suitable for use as an Adjunct Culture or in the production of Long-ripened Cheeses requiring gradual acidification, as it prevents over-acidification during the initial stages [36]. Furthermore, the study by Barzegar et al. (2021) demonstrated that one *L. casei* strain exhibited a stronger acidifying activity in reconstituted skim milk; this strain reported a pH drop of approximately 0.44 at 6 hours and a final pH drop of about 1.57 after 24 h of incubation, placing it at the upper limit of the moderate acidifiers category [37]. Similarly, research by Davati et al. (2016) reported considerable variability in the acidification rates of LAB strains, with their pH drops (ΔpH) after 24 hours spanning a range of 1.42 to 1.93 [19]. The discrepancies observed in acidification rates among different LAB species are linked to the strains' efficiency in breaking down carbon and nitrogen substrates in the medium. These differences encompass their capacity for effective assimilation of essential growth nutrients, as well as the presence or absence of specialized membrane transport systems, all of which directly influence acid production kinetics [38].

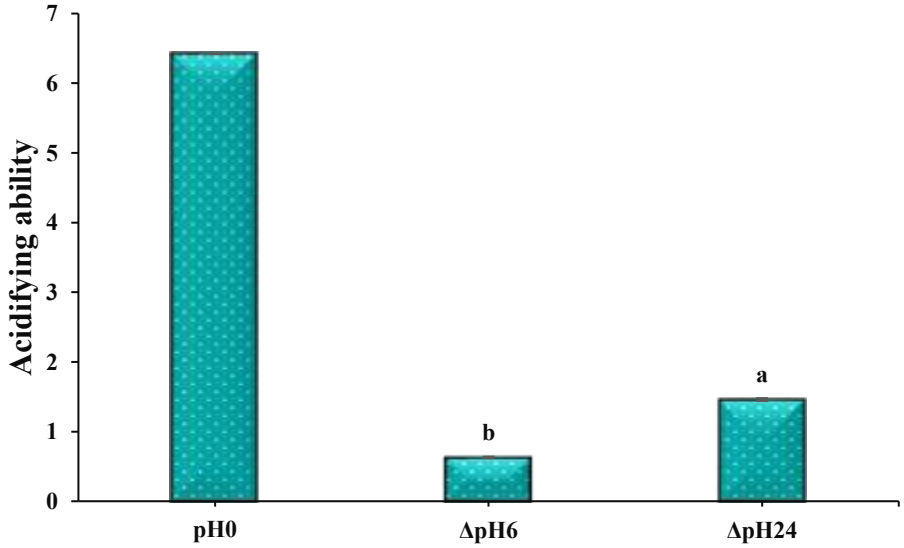

Fig.2. Acidifying activity of the strain *L. casei* ANNC 26. The initial pH (pH0) and the amount of pH reduction after 6 hours (ΔpH6) and 24 hours (ΔpH24) are shown. Different letters indicate significant differences ($p < 0.05$)**.**

3.4. Proteolysis activity

Proteolytic activity is a fundamental enzymatic process absolutely essential for the optimal development of organoleptic (sensory) properties in fermented milk products. The production of high-quality fermented dairy products is directly dependent on the efficiency of the starter bacteria's proteolytic systems; the hydrolysis of proteins leads to the formation of peptides and free amino acids. These compounds not only directly impact the product's flavor profile and texture but also serve as vital flavor and aroma precursors during the ripening process [38]. The proteolytic activity of the *L. casei* ANNC 26 strain under investigation was evaluated by measuring the diameter of the clear zone created around the well. The criterion for determining a strain's proteolytic activity, based on the method proposed by Vuillemard et al. (1986), stipulates that a strain is considered proteolytic if its lysis zone diameter falls within the range of 15 to 21 mm [39]. This activity was reported for the studied strain by the formation of a casein hydrolysis halo with a mean diameter of 21.69 ± 0.09 mm (Fig. 3). The process of proteolysis is fundamentally dependent on the bacterial proteinase system. In all LAB strains, a cell-envelope proteinase has been identified, which is situated in the cell wall and whose catalytic function is the hydrolysis of extracellular proteins and the subsequent release of peptides into the medium [40]. These results align with findings from other studies; for instance, Hawaz et al. (2016) reported the proteolytic halo diameters of their studied strains to be in the range of 15 to 21 mm, while Barzegar et al. (2021) recorded an even higher proteolytic halo diameter for a *L. casei* strain, equivalent to 23 mm, which confirms the

intraspecies diversity and high activity of the *L. casei* species [37.38]. Beyond flavor and texture, proteolytic activity leads to the release of bioactive peptides that possess positive nutritional effects, including antioxidant, antibacterial, antihypertensive, and immune-system boosting properties [41]. These functional characteristics have resulted in a focused interest today on the controlled use of proteolytic enzymes in the design of functional dairy products. Furthermore, in fermented products such as yogurt and kefir, proteolysis plays a role in improving digestibility, reducing the allergenicity of milk proteins, and creating specific flavor profiles [42]. The control of this process through the selection of appropriate enzymes, optimized processing conditions, and specific microbial strains is one of the key strategies for enhancing the quality and nutritional value of dairy products [43].

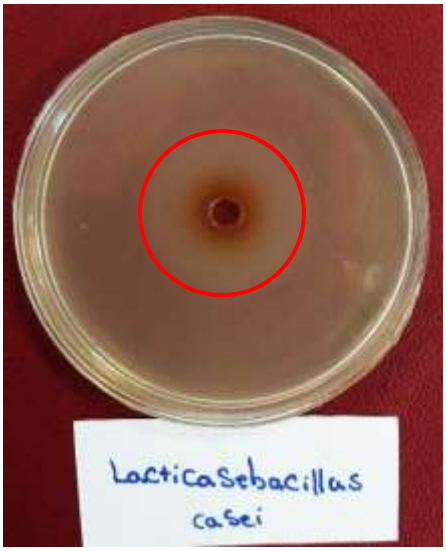

Fig. 3. Proteolytic activity of *L. casei* ANNC 26.

3.5. Autolytic activity

Autolysis, as a physiological state in bacteria under stress, constitutes a vital process in fermented products, during which cell lysis (cell breakdown) is initiated by the activity of peptidoglycan hydrolases. The primary biochemical consequence of LAB cell lysis is the release of intracellular peptidases that cleave peptides into smaller units and free amino acids. Consequently, key aromatic precursors (such as branched-chain amino acids and sulfur-containing compounds) are substantially released into the medium, playing a pivotal role in the formation of desired volatile flavor compounds [44,45]. Based on the Lysis Rate, LAB strains are distinctly categorized into three groups by the intensity of their Autolytic Activity: weak activity refers to lysis of less than 14%; moderate activity is observed in the range of 15% to 24%; and good activity is characterized by a lysis rate between 25% to 65%. The autolysis results for the *L. casei* ANNC 26 strain are shown in Fig 4. The autolytic activity of the investigated *L. casei* ANNC 26 strain was significantly different across various temperatures ($p < 0.05$). The highest cell lysis was observed at the optimum growth temperature (37 °C), registering 57.21±0.64%, which classifies this strain in the good autolytic activity range. With a decrease in temperature, the lysis rate dropped sharply; at refrigeration temperature (4 °C), autolysis decreased to 22.11±0.33%, classifying the strain as moderate. The lowest lysis was recorded at freezing temperature (−20 °C), with a value of 16.32 ± 0.84%. These findings are consistent with other studies; for instance, Barzegar et al. (2021) reported their studied *L. casei* strain had moderate autolytic activity with a lysis rate of 15% [38]. Similarly, Davati et al. (2016), in a study on LAB strains isolated from milk, argued that all investigated strains exhibited a good level of autolytic activity [19]. The level of LAB autolysis is a process dependent on environmental and physiological factors, being significantly influenced by parameters such as carbon source, temperature, osmotic concentration, growth phase, and pH [45]. From a quality and processing perspective, cultures exhibiting high autolytic intensity are of particular importance because they facilitate the faster release of lipolytic and proteolytic enzymes, which effectively contributes to the flavor development of

fermented dairy products. However, it must be considered that excessive lysis of starter LAB can lead to undesirable consequences, negatively affecting the final cheese quality by reducing its fermentative capacity.

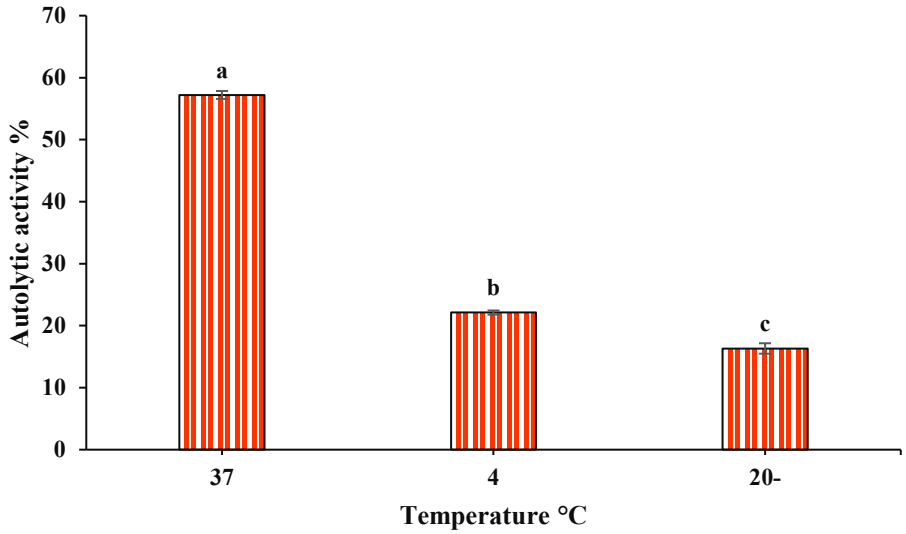

Fig. 4. Autolytic Activity of *L. casei* ANNC 26. at Different Temperatures (37 °C, 4 °C, and -20 °C). Different letters indicate significant differences ($p < 0.05$)

4-Conclusion

The results from the evaluation of the technological properties of the indigenous *L. casei* ANNC 26 strain isolated from Khiki cheese indicate the high potential of this strain for industrial application. This strain possesses acceptable stability at processing temperatures and demonstrates a high capacity for EPS production, which significantly aids in improving the product's consistency and water retention. Its moderate acidifying activity makes it highly suitable for use as an Adjunct Starter Culture in long-ripened cheeses, preventing rapid over-acidification. The strain's outstanding enzymatic activities, proteolysis and autolysis (with cell lysis up to 57.21%), ensure the effective release of flavor precursors and bioactive peptides. Collectively, the unique combination of these desirable traits transforms the indigenous *L. casei* ANNC 26 strain into a promising and ideal candidate for enhancing the sensory quality, texture, and stability of Khiki cheese, thereby paving the way for the successful development and industrialization of this traditional product.

Data Availability

All data relevant to the study are included in the article.

Conflict of Interest

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

Funding Statement

Not applicable.

Authors Contributions

Behrooz Alizadeh Behbahani: Review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Parisa Ghasemi:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Alireza Vasiee**: Writing –review & editing, Supervision, Methodology, Investigation. **Zeinab Mousavi**: Visualization, Methodology, Investigation, Formal analysis.

Consent for publication

All authors approved the manuscript for publication.

Ethical approval

This article does not contain any studies with human or animal subjects.

Clinical trial number

not applicable.

Consent to Participate declaration

not applicable.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used "Microsoft Copilot" in order to paraphrase and grammatically check the sentences. After using this tool/service, the authors reviewed

and edited the content as needed and take full responsibility for the content of the published article.

Acknowledgments

The authors wish to express their profound gratitude sincerely to the Research Deputy of Agricultural Sciences and Natural Resources University of Khuzestan for financially supported this project (1403.27).

5-References

[1] Marco, M. L., Sanders, M. E., Gänzle, M., Arrieta, M. C., Cotter, P. D., De Vuyst, L., Hill, C., Holzapfel, W., Lebeer, S., Merenstein, D., Reid, G., Wolfe, B. E., & Hutkins, R. (2017). The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nature Reviews Gastroenterology & Hepatology*, 14(3), 196–208.

[2] Rezac, S., Kok, C. R., Heermann, M., & Hutkins, R. (2018). Fermented foods as a dietary source of live organisms. *Frontiers in Microbiology*, 9, 1785.

[3] Liu, W., Zhang, R., Shuai, Y., Li, B., Wang, J., & He, X. (2019). Role of lactic acid bacteria in fermented foods and their health benefits. *Food Science and Human Wellness*, 8(2), 103–109.

[4] Tamang, J. P., Cotter, P. D., Endo, A., Han, N. S., Kort, R., Liu, S. Q., Mayo, B., Westerik, N., & Hutkins, R. (2020). Fermented foods in a global age: East meets West. *Comprehensive Reviews in Food Science and Food Safety*, 19(1), 184–217.

[5] LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., & Ventura, M. (2017). Bacteria as vitamin suppliers to their host: A gut microbiota perspective. *Current Opinion in Biotechnology*, 44, 16–23.

[6] Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., Morelli, L., Canani, R. B., Flint, H. J., Salminen, S., Calder, P. C., & Sanders, M. E. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, 11(8), 506–514.

[7] Hayaloglu, A. A. (2014). Cheese varieties ripened under brine: A review. *Trends in Food Science & Technology*, 41(2), 226–259.

[8] Hayaloglu, A. A., & Farkye, N. Y. (2011). Cheese with brine: White brined cheeses. In J. W. Fuquay (Ed.), *Encyclopedia of Dairy Sciences* (2nd ed., pp. 783–789). Academic Press.

[9] Randazzo, C. L., Todaro, A., Pino, A., Pitino, I., Corona, O., Caggia, C., & Di Bella, S. (2018). Microbiota and metabolome during controlled and spontaneous fermentation of Nocellara Etnea table olives. *Food Microbiology*, 73, 136–146.

[10] Zago, M., Fornasari, M. E., Carminati, D., Burns, P., Suàrez, V., Vinderola, G., Reinheimer, J., & Giraffa, G. (2011). Characterization and probiotic potential of *Lactobacillus plantarum* strains isolated from cheeses. *Food Microbiology*, 28(5), 1033–1040.

[11] Linares, D. M., Gómez, C., Renes, E., Fresno, J. M., Tornadijo, M. E., Ross, R. P., & Stanton, C. (2017). Lactic acid bacteria and bifidobacteria with potential to design natural biofunctional health-promoting dairy foods. *Frontiers in Microbiology*, 8, 846.

[12] Alizadeh Behbahani, B., & Noshad, M. (2021). Isolation and Identification of Lactobacillus Strains from Behbahan Local Cheeses and Investigation of Technological and Antimicrobial Properties of These Strains against Major Food Pathogens. *Iranian Journal of Nutrition Sciences and Food Technology*, *16*(1), 133-142.

[13] Dimitrellou, D., Kandylis, P., & Kourkoutas, Y. (2019). Thermal tolerance of probiotic microorganisms in functional dairy products: A review. *Foods*, 8(10), 431.

[14] García-Cano, I., Rocha-Mendoza, D., Ortega-Anaya, J., Wang, K., Kosmerl, E., & Jiménez-Flores, R. (2019). Lactic acid bacteria isolated from dairy products as potential producers of EPS and their effect on textural properties of fresh cheese. *Food Microbiology*, 84, 103247.

[15] Kulkarni, S., Haq, S. F., Samant, S., & Sukumaran, S. (2018). Adaptation of *Lactobacillus acidophilus* to thermal stress yields a thermotolerant variant which also exhibits improved survival at pH 2. *Probiotics and antimicrobial proteins*, *10*(4), 717-727.

[16] Ogunremi, O. R., Leischtfeld, S. F., & Schwenninger, S. M. (2022). MALDI-TOF MS profiling and exopolysaccharide production properties of lactic acid bacteria from Kunu-zaki-A cereal-based Nigerian fermented beverage. *International Journal of Food Microbiology*, *366*, 109563.

[17] Xanthopoulos, V., Hatzikamari, M., Adamidis, T., Tsakalidou, E., Tzanetakis, N., & Litopoulou-Tzanetaki, E. (2000). Heterogeneity of *Lactobacillus plantarum* isolates from Feta cheese throughout ripening. *Journal of Applied Microbiology*, 88(6), 1056-1064.

[18] Saci, A., Gharbi, S., Djadouni, F., & Karkachi, N. (2025). Evaluation of selected functional traits in lactic acid bacteria isolated from

Algerian Makatia goat milk for food applications. *Acta Biologica Slovenica*, *68*(4).
[19] Davati, N., Tabatabaee, Y. F., Zibaee, S., Shahidi, F., & Edalatian, M. R. (2016). Isolation and identification of Lactobacillus bacteria from raw milk of Iranian one humped camel (Camelus dromedarius) and evaluation of their technological properties.
[20] Ferrando, V., Quiberoni, A., Reinhemer, J., & Suárez, V. (2015). Resistance of functional Lactobacillus plantarum strains against food stress conditions. *Food microbiology*, *48*, 63-71.
[21] Wang, Y., Hao, F., Lu, W., Suo, X., Bellenger, E., Fu, N., ... & Chen, X. D. (2020). Enhanced thermal stability of lactic acid bacteria during spray drying by intracellular accumulation of calcium. *Journal of Food Engineering*, *279*, 109975.
[22] Gouesbet, G., Jan, G., & Boyaval, P. (2001). Lactobacillus delbrueckii ssp. bulgaricus thermotolerance. *Le Lait*, *81*(1-2), 301-309.
[23] Chen, X., Liu, Y., Fan, M., Wang, Z., Wu, W., & Wang, J. (2017). Thermal and chemical inactivation of Lactobacillus virulent bacteriophage. *Journal of Dairy Science*, *100*(9), 7041-7050.
[24] Noshad, M., Alizadeh Behbahani, B., & Hojjati, M. (2021). Investigation of probiotic and technological characteristics of lactic acid bacteria isolated from native Doogh of Behbahan. *Food Research Journal*, *31*(4), 169-186.
[25] Jurášková, D., Ribeiro, S. C., & Silva, C. C. (2022). Exopolysaccharides produced by lactic acid bacteria: from biosynthesis to health-promoting properties. *Foods*, *11*(2), 156.
[26] Santal, A. R., Singh, N. P., & Singha, T. K. (2019). Characterization of extracellular polymeric substance producing isolates from wastewaters and their antibacterial prospective. *J. Appl. Biol. Biotech*, *7*, 56-62.
[27] Bacosa, H. P., Kamalanathan, M., Chiu, M. H., Tsai, S. M., Sun, L., Labonté, J. M., ... & Quigg, A. (2018). Extracellular polymeric substances (EPS) producing and oil degrading bacteria isolated from the northern Gulf of Mexico. *PLoS One*, *13*(12), e0208406.
[28] Minari, G. D., Piazza, R. D., Sass, D. C., & Contiero, J. (2024). EPS production by Lacticaseibacillus casei using glycerol, glucose, and molasses as carbon sources. *Microorganisms*, *12*(6), 1159.
[29] Cerning, J. C. M. C., Renard, C. M. G. C., Thibault, J. F., Bouillanne, C., Landon, M., Desmazeaud, M., & Topisirovic, L. (1994). Carbon source requirements for exopolysaccharide production by Lactobacillus casei CG11 and partial structure analysis of the polymer. *Applied and Environmental Microbiology*, *60*(11), 3914-3919.
[30] Coda, R., Di Cagno, R., Gobbetti, M., & Rizzello, C. G. (2014). Sourdough lactic acid bacteria: Exploration of non-wheat cereal-based fermentation. *Food microbiology*, *37*, 51-58.
[31] Jialiang, Z., Huaxi, Y., Xianjun, L., Wen, Z., & Xiaodong, X. (2015). Production of exopolysaccharides by *Lactobacillus fermentum* F6 isolated from sourdough and its application in gluten-free bread. *Food Hydrocolloids, 43*, 182–188.
[32] Paul, D., Kumari, P. K., & Siddiqui, N. (2023). Yeast carotenoids: Cost-effective fermentation strategies for health care applications. *Fermentation*, *9*(2), 147.
[33] Lin, P. C., Wu, D. T., Xie, J., Zhao, J., & Li, S. P. (2015). Characterization and comparison of bioactive polysaccharides from the tubers of Gymnadenia conopsea. *Food Hydrocolloids*, *43*, 199-206.
[34] Rahmati, F. (2017). Characterization of Lactobacillus, Bacillus and Saccharomyces isolated from Iranian traditional dairy products for potential sources of starter cultures. *AIMS microbiology*, *3*(4), 815.
[35] Mohammed, S., & Çon, A. H. (2021). Isolation and characterization of potential probiotic lactic acid bacteria from traditional cheese. *Lwt*, *152*, 112319.
[36] Iqbal, M. W., Mu, W., Khan, I. M., Mohsin, A., Rehman, A., & Koko, M. Y. F. (2017). Development of probiotic soft cheese with Lactobacillus casei as adjunct culture. *Journal of Academia and Industrial Research (JAIR)*, *6*(1), 1.
[37] Barzegar, H., Alizadeh Behbahani, B., & Falah, F. (2021). Safety, probiotic properties, antimicrobial activity, and technological performance of Lactobacillus strains isolated from Iranian raw milk cheeses. *Food Science & Nutrition*, *9*(8), 4094-4107.
[38] Hawaz, E., Guesh, T., Kebede, A., & Menkir, S. (2016). Characterization of lactic acid bacteria from camel milk and their technological properties to use as a starter culture. *East African Journal of Sciences*, *10*(1), 49-60.
[39] Vuillemard, J. C., Amiot, J., & Gauthier, S. (1986). Evaluation de l'activité protéolytique de bactéries lactiques par une méthode de diffusion sur plaque. *Microbiol. Alim. Nutr*, *3*, 327-332.
[40] Namdari, A., & Nejati, F. (2016). Development of Antioxidant Activity during Milk Fermentation by Wild Isolates of *Lactobacillus helveticus*. *Applied Food Biotechnology*, *3*(3), 178–186.
[41] El-Salam, M. H. A., & El-Shibiny, S. (2017). Bioactive peptides of buffalo, camel, goat, sheep, mare, and yak milks and milk products. *Food Reviews International*, 33(1), 1–29.

[42] Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017). *Fundamentals of Cheese Science* (2nd ed.). Springer.
[43] Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2011). Microstructure and composition of full fat Cheddar cheese made with ultrafiltered milk retentate. *Food Research International*, 44(1), 91–98.
[44] Xu, Y., Wang, T., Kong, J., & Wang, H. L. (2015). Identification and functional characterization of AclB, a novel cell-separating enzyme from Lactobacillus casei. *International Journal of Food Microbiology*, *203*, 93-100.
[45] Dalca, S., Simsek, Ö., Gursoy, O., & Yilmaz, Y. (2018). Selection of autolytic lactic acid bacteria as potential adjunct cultures to accelerate ripening of white-brined cheeses. *Mljekarstvo*, *68*(4).

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

ارزیابی ویژگی‌های تکنولوژیکی سویه *Lacticaseibacillus casei* ANNC 26 جداشده از پنیر سنتی خیکی به عنوان یک کشت آغازگر کمکی

بهروز علیزاده بهبهانی[*۱]، پریسا قاسمی[۲]، علیرضا وسیعی[۳]، زینب موسوی[٤]

۱- دانشیار، گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

۲- دانشجوی دکتری، گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

۳- استادیار، گروه پژوهشی ایمنی و کنترل کیفیت مواد غذایی، مؤسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران

۴- دانشجوی کارشناسی ارشد، گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰٤/۱۰/۱٤

تاریخ داوری: ۱٤۰٤/۱۱/۱۲

تاریخ پذیرش: ۱٤۰٤/۱۱/۱۹

کلمات کلیدی:

پنیر خیکی سنتی،

پلی‌ساکارید خارج‌سلولی،

قابلیت اسیدی شدن،

Lacticaseibacillus casei.

DOI: 10.48311/fsct.2026.118719.83022

* مسئول مکاتبات:

B.alizadeh@asnrukh.ac.ir

چکیده

بقا و کارایی عملکردی کشت‌های آغازگر در محیط‌های فرآوری صنعتی غذا، وابستگی حیاتی به مقاومت آن‌ها در برابر تنش‌ها، به‌ویژه دمای بالا، دارد. این مطالعه به ارزیابی جامع خواص تکنولوژیکی کلیدی سویه بومی *Lacticaseibacillus casei* ANNC 26 جداشده از پنیر سنتی خیکی شامل مقاومت حرارتی، توانایی تولید پلی‌ساکارید خارج‌سلولی (EPS)، قابلیت اسیدی شدن و فعالیت‌های آنزیمی (پروتئولیتیکی و اتولیتیکی) پرداخت. ارزیابی مقاومت حرارتی نشان داد سویه پایداری قابل توجهی در برابر تنش دارد. بالاترین بقا (۵۷/٤۲ ٪) در ۵۰ درجه سانتی‌گراد ثبت شد و حتی در ۸۰ درجه سانتی‌گراد نیز بقای ۲۵/۷۳ ٪ مشاهده شد که نشان‌دهنده انعطاف‌پذیری آن در شرایط حرارتی است. این سویه توانایی قابل توجهی در سنتز EPS نشان داد که به‌ویژه توسط ساکارز و گلوکز القا شد و پتانسیل آن را به عنوان یک افزودنی زیستی چندمنظوره برای بهبود بافت و نگهداری آب تأیید می‌کند. با فعالیت اسیدی شدن (ΔpH_{24h}=۱/٤۸)، این سویه به عنوان یک اسیدی‌کننده متوسط طبقه‌بندی شد و برای استفاده به عنوان کشت کمکی در پنیرهای با دوره رسیدن طولانی که نیاز به اسیدی شدن تدریجی دارند، بسیار مناسب است. علاوه بر این، سویه مورد بررسی فعالیت پروتئولیتیکی بالا (قطر هاله ۰/۰۹ ± ۲۱/٦۹ میلی‌متر) و فعالیت اتولیتیکی "خوب" از خود نشان داد. این فعالیت‌های آنزیمی بالا، پتانسیل آن را برای آزادسازی پیش‌سازهای طعمی و پپتیدهای زیست‌فعال تأیید می‌کند. در مجموع، این ویژگی‌های مطلوب، سویه بومی *L. casei* ANNC 26 را به عنوان کاندیدای امیدوارکننده برای کاربرد هدفمند به عنوان کشت کمکی آغازگر، جهت بهبود قابل توجه بافت، طعم و پایداری پنیر خیکی و توسعه صنعتی این محصول سنتی، تأیید می‌کند.