Advances in Bioresearch
Adv. Biores., Vol 17 (3) March 2026:101-107

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.17.3.101107

Advances in Bioresearch

ORIGINAL ARTICLE

Screening and characterization of probiotic lactic acid bacteria from fermented Toddy palm juice

Nissam Sainudeen[1], Divya Vattoly[1], Yogananth, N[1]., Syed Ali, M[2]and Anuradha, V[3]
[1]Dept of Microbiology, Mohamed Sathak College of Arts and Science, Chennai, India
[2]Dept of Biotechnology, Mohamed Sathak College of Arts and Science, Chennai, India
[3]Dept of Biochemistry, Mohamed Sathak College of Arts and Science, Chennai, India

ABSTRACT
Naturally fermented toddy palm (Borassus flabellifer) juice is a traditional beverage widely consumed in South India, however, its probiotic microbiota remains poorly explored. The present study aimed to isolate and evaluate lactic acid bacteria (LAB) with probiotic potential from fermented toddy palm juice. Microbial analysis revealed a predominant LAB population (7.65 ± 0.06 log CFU/mL), followed by total bacterial count (7.48 ± 0.10 log CFU/mL) and yeast count (3.72 ± 0.32 log CFU/mL). Ten LAB isolates were obtained, among which three isolates exhibited strong tolerance to acidic pH (2.5) and 0.3% bile salts. The selected isolates demonstrated notable antimicrobial activity against common foodborne pathogens, including Escherichia coli, Salmonella typhi, Vibrio parahaemolyticus, Pseudomonas sp, Bacillus cereus, and Staphylococcus aureus, with inhibition zones ranging from 14 to 25 mm. All isolates showed bile salt hydrolase activity and were non-hemolytic and DNase-negative, indicating safety. Additionally, amylolytic activity and exopolysaccharide production were observed. These findings suggest that fermented toddy palm juice is a promising source of probiotic LAB for functional beverage applications.
Keywords: *Toddy palm juice, Lactic acid bacteria, Probiotics, Characterization.*

Received 05.02.2026 Revised 19.02.2026 Accepted 24.03.2026

How to cite this article:
Nissam S, Divya V, Yogananth, N., Syed Ali, Mand Anuradha, V. Screening and characterization of probiotic lactic acid bacteria from fermented Toddy palm juice. Adv. Biores., Vol 17 (3) March 2026: 101-107.

INTRODUCTION

Fermented foods have long been an essential part of human diets, contributing not only to food preservation but also to nutritional enhancement and health promotion. Central to many fermentation processes are lactic acid bacteria (LAB), a diverse group of Gram-positive microorganisms that metabolize carbohydrates into organic acids and other bioactive compounds. These metabolic activities improve food safety, extend shelf life, and enhance sensory attributes [9]. In recent years, LAB have gained considerable attention for their role as probiotics, defined as live microorganisms that, when consumed in adequate amounts, confer health benefits to the host [6]. Probiotic LAB are known to exert multiple beneficial effects, including modulation of gut microbiota, inhibition of pathogenic microorganisms, strengthening of intestinal barrier function, and immune regulation [7]. These effects are highly strain-specific and depend on the ability of LAB to survive gastrointestinal stresses such as low pH and bile salts, adhere to intestinal epithelial cells, and produce antimicrobial substances [9]. As a result, there is growing interest in isolating novel probiotic strains from traditional fermented foods, which often harbor diverse and resilient microbial communities. Traditional fermented beverages represent an important but underexplored source of indigenous probiotic LAB. Unlike industrially produced fermented foods, traditional beverages rely on spontaneous fermentation driven by naturally occurring microbiota associated with raw materials and local environments [10]. Such products are particularly valuable in the development of non-dairy probiotics, catering to populations with lactose intolerance or limited access to dairy products [11].

Toddy palm juice, obtained from the sap of the Palmyra palm (*Borassus flabellifer*), is a traditional fermented beverage widely consumed in South India and other tropical regions. Fresh palm sap is rich in fermentable sugars, amino acids, vitamins, and minerals, providing a favorable substrate for microbial

growth and fermentation [8]. Natural fermentation of toddy palm sap results in the formation of organic acids and other metabolites that influence its microbial stability and potential health benefits. Despite its widespread consumption, scientific studies focusing on the probiotic LAB present in fermented toddy palm juice remain limited. The microbial composition of fermented palm sap is influenced by factors such as palm species, geographic location, tapping methods, and fermentation conditions. LAB commonly reported in palm sap and similar plant-based fermentations include members of the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Enterococcus* [4, 5]. These bacteria contribute to fermentation stability through acidification and the production of antimicrobial compounds.

In this context, the present study focuses on the isolation and characterization of probiotic LAB from fermented toddy palm juice, aiming to explore its potential as a non-dairy functional beverage and a source of beneficial probiotic strains.

MATERIAL AND METHODS

Sample collection and fermentation

Fresh toddy palm juice was obtained from naturally tapped palmyra palm (*Borassus flabellifer*) trees from selected local sources in Chennai, Tamil Nadu, India. Sap collection was carried out under hygienic conditions using sterile containers. The samples were immediately sealed, transported to the laboratory in insulated ice boxes, and processed within 4 h to minimize changes in microbial composition. Natural fermentation was allowed to continue at room temperature (28–30°C) without the addition of starter cultures to preserve indigenous microbiota.

Enumeration of microbial population

Microbial enumeration was performed using standard plate count techniques. Ten milliliters of fermented toddy palm juice was aseptically transferred into 90 mL of sterile phosphate-buffered saline (PBS, pH 7.2) and homogenized thoroughly. Serial dilutions were prepared up to 10^{-7}. Total viable bacterial counts were determined on Plate Count Agar incubated at 37°C for 48 h. Lactic acid bacteria were selectively enumerated on MRS agar supplemented with 0.5% calcium carbonate and incubated anaerobically at 37°C for 24–48 h. Yeast populations were enumerated on Potato Dextrose Agar containing chloramphenicol (100 mg/L) after incubation at 28°C for 72 h. Colony counts were expressed as log CFU/mL.

Isolation and maintenance of LAB

Colonies exhibiting typical LAB morphology and clear halos on MRS-$CaCO_3$ agar were selected and purified through repeated streaking. Pure cultures were maintained on MRS agar slants at 4 °C and periodically subcultured to ensure viability. Preliminary characterization of isolates included Gram staining, catalase reaction, cell morphology, motility testing, and carbohydrate fermentation profiles following standard microbiological protocols.

Evaluation of probiotic properties

Acid and bile salt tolerance

To assess acid tolerance, LAB cultures grown overnight in MRS broth were harvested by centrifugation at 5,000 rpm for 10 min and washed twice with sterile PBS. The cell pellets were resuspended in MRS broth adjusted to pH 2.5 using 1 N HCl and incubated at 37 °C. For bile tolerance, washed cells were inoculated into MRS broth supplemented with 0.3% (w/v) ox bile. Viable counts were determined at 0 and 3 h by plating on MRS agar, and survival rates were calculated.

Hydrolase activity

Hydrolase activity was determined by streaking LAB isolates onto MRS agar supplemented with 0.5% bile salts and 0.37 g/L calcium chloride. Plates were incubated anaerobically at 37 °C for 72 h. The formation of opaque precipitate zones around colonies was considered indicative of positive BSH activity.

Antimicrobial activity

Antimicrobial potential of LAB isolates was evaluated using the agar well diffusion method. Cell-free supernatants were obtained by centrifugation of overnight cultures at 8,000 rpm for 15 min. Indicator foodborne pathogens, including *Escherichia coli*, *Salmonella typhi*, *Vibrio parahaemolyticus*, *Pseudomonas* sp., *Bacillus cereus*, and *Staphylococcus aureus*, were grown to mid-log phase and uniformly spread on nutrient agar plates. Wells of 6 mm diameter were filled with 100 µL of supernatant, and plates were incubated at 37 °C for 24 h. Zones of inhibition were measured and recorded.

Safety assessment

Safety characteristics of LAB isolates were evaluated by hemolysis and DNase tests. Hemolytic activity was assessed by streaking isolates onto 5% sheep blood agar plates and observing hemolysis patterns after incubation at 37 °C for 48 h. DNase activity was determined using DNase agar plates, followed by the addition of 1 N HCl to visualize clear zones around colonies.

Assessment of technological properties

Amylolytic activity

Amylolytic activity of the selected lactic acid bacteria (LAB) isolates was evaluated to determine their ability to hydrolyze starch, an important technological trait for fermentation of plant-based substrates. LAB isolates were streaked onto MRS agar plates supplemented with 1% (w/v) soluble starch and incubated at 37 °C for 48 h under anaerobic conditions. Following incubation, the plates were flooded with freshly prepared iodine solution and allowed to react for 2–3 min. Excess iodine was gently drained off, and the plates were examined for the presence of clear zones surrounding the bacterial colonies. The formation of distinct halos indicated starch hydrolysis due to extracellular amylase activity.

Exopolysaccharide (EPS) Production

Exopolysaccharide production was assessed to evaluate the ability of LAB isolates to synthesize extracellular polysaccharides that can improve texture, viscosity, and mouthfeel of fermented products. LAB isolates were streaked onto MRS agar plates supplemented with 2% (w/v) sucrose and incubated at 37 °C for 48–72 h. After incubation, colonies were examined visually for mucoid or ropy characteristics, which are indicative of EPS production.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation. Data analysis was performed using standard descriptive statistical methods.

RESULTS

Microbial profile of fermented toddy palm juice

The microbiological analysis of naturally fermented toddy palm juice revealed a diverse microbial population dominated by lactic acid bacteria (LAB). As shown in Table 1, LAB constituted the predominant group with a mean count of 7.65 ± 0.06 log CFU/mL. The total viable bacterial count was slightly lower at 7.48 ± 0.10 log CFU/mL, indicating that LAB represented the major bacterial population in the fermented juice. Yeast populations were also detected, though at comparatively lower levels (3.72 ± 0.32 log CFU/mL), suggesting their supportive but non-dominant role during fermentation.

Table 1. Microbial counts in fermented toddy palm juice

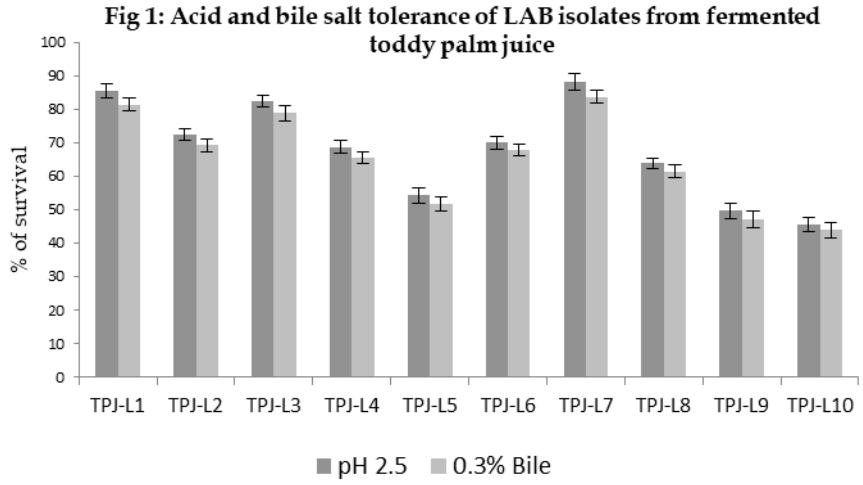
Microbial group	Count (log CFU/mL)
Total viable bacteria	7.48 ± 0.10
Lactic acid bacteria	7.65 ± 0.06
Yeasts	3.72 ± 0.32

Isolation and preliminary characterization of LAB

A total of ten LAB isolates were obtained from MRS-$CaCO_3$ agar plates based on colony morphology and clear zone formation. All isolates were Gram positive, catalase negative, non-motile, and rod and cocci shaped, confirming their identity as LAB. These isolates were further screened for probiotic properties.

Acid and bile salt tolerance of LAB Isolates

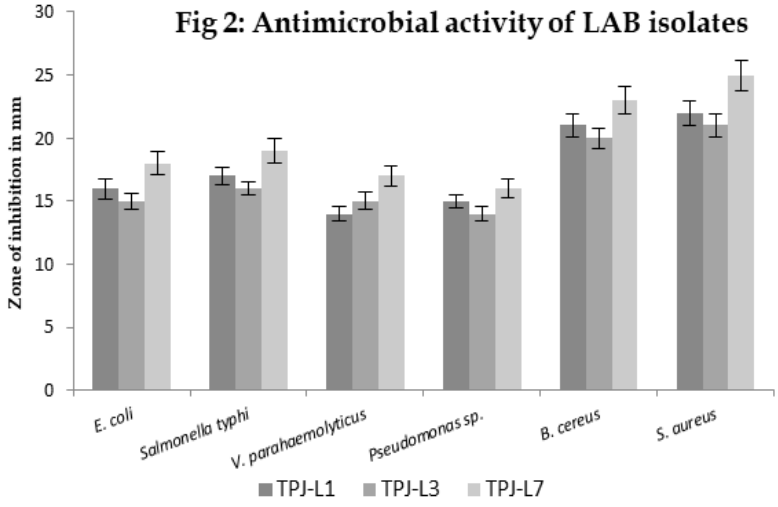
The ability of lactic acid bacteria (LAB) isolates to tolerate acidic pH and bile salts was evaluated as a key criterion for probiotic potential. All ten isolates (TPJ-L1 to TPJ-L10) were subjected to pH 2.5 and 0.3% bile salt conditions for 3 h, and their survival rates were determined based on viable counts. As shown in Fig 1, marked variations in tolerance levels were observed among the isolates. Three isolates, namely TPJ-L1, TPJ-L3, and TPJ-L7, exhibited strong resistance to acidic conditions, retaining more than 80% viability after 3 h of exposure. Moderate acid tolerance (60–75% survival) was observed in four isolates (TPJ-L2, TPJ-L4, TPJ-L6, and TPJ-L8), whereas the remaining three isolates showed comparatively lower survival rates under acidic stress.



Values are expressed as mean ± standard deviation (n = 3).

Antimicrobial activity against foodborne pathogens

The antimicrobial potential of the selected LAB isolates was evaluated against common foodborne pathogens. All three isolates demonstrated inhibitory activity against both Gram-positive and Gram-negative bacteria. As shown in Fig 2, inhibition zones ranged from 14 to 25 mm. TPJ-L7 exhibited the strongest antimicrobial activity, particularly against *Staphylococcus aureus* (25 mm) and *Bacillus cereus* (23 mm). Moderate inhibition was observed against *Escherichia coli*, *Salmonella typhi*, *Vibrio parahaemolyticus*, and *Pseudomonas* sp., highlighting the broad-spectrum antagonistic potential of the isolates.



Values are expressed as mean ± standard deviation (n = 3).

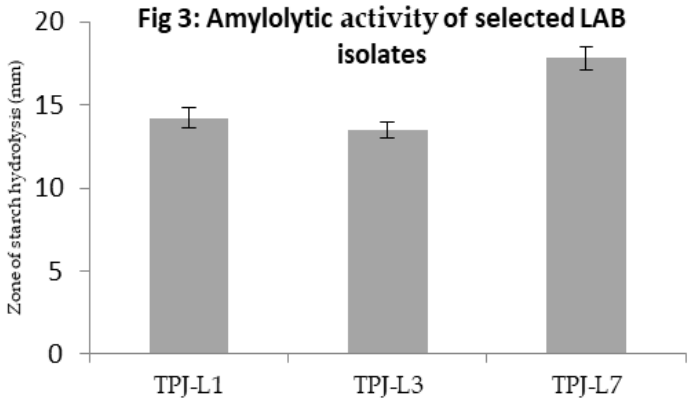
Bile salt hydrolase activity and safety assessment

All three selected LAB isolates showed positive bile salt hydrolase activity, indicated by the formation of precipitation zones on bile salt-supplemented media. Safety evaluation revealed that all isolates were non-hemolytic on blood agar and DNase-negative, confirming their non-pathogenic nature.

Technological properties

Amylolytic activity

The amylolytic activity of the selected lactic acid bacteria (LAB) isolates was assessed to evaluate their ability to hydrolyze starch. All three selected isolates (TPJ-L1, TPJ-L3, and TPJ-L7) exhibited positive amylolytic activity on starch-supplemented MRS agar plates. After incubation and iodine staining, distinct clear zones were observed around the colonies, confirming extracellular starch degradation. Quantitative assessment of starch hydrolysis revealed variation in the extent of amylase activity among the isolates (Fig 3). TPJ-L7 produced the largest zone of clearance, indicating comparatively higher amylolytic potential. TPJ-L1 and TPJ-L3 showed moderate but clearly defined hydrolysis zones, demonstrating effective starch utilization capability.



Values are expressed as mean ± standard deviation (n = 3).

Exopolysaccharide (EPS) Production

Exopolysaccharide (EPS) production by the selected lactic acid bacteria (LAB) isolates was evaluated and all three selected isolates (TPJ-L1, TPJ-L3, and TPJ-L7) exhibited positive EPS production when grown on sucrose-supplemented MRS agar. Colonies displayed distinct mucoid and ropy characteristics, indicating the secretion of extracellular polysaccharides. Qualitative differences in EPS production were observed among the isolates based on colony appearance and ropiness intensity. TPJ-L7 exhibited pronounced ropy and highly mucoid colonies, suggesting higher EPS-producing potential. TPJ-L1 showed moderate mucoid characteristics with visible ropiness, while TPJ-L3 exhibited slightly less pronounced but clearly detectable EPS production. The formation of viscous threads upon gentle lifting of colonies with a sterile inoculating loop further confirmed EPS synthesis.

Table 2. Exopolysaccharide (EPS) production by selected LAB isolates

Isolate	Colony morphology on sucrose-MRS agar	Ropiness	EPS production level
TPJ-L1	Moderately mucoid	Present	Moderate
TPJ-L3	Slightly mucoid	Present	Moderate
TPJ-L7	Highly mucoid	Strong	High

EPS production level was assessed qualitatively based on colony mucoidity and ropiness intensity.

DISCUSSION

Traditional fermented beverages are increasingly recognized as valuable sources of probiotic microorganisms due to their rich and diverse microbial communities. In the present study, naturally fermented toddy palm juice (*Borassus flabellifer*) was investigated as a potential reservoir of probiotic lactic acid bacteria (LAB), and the results provide important insights into its microbiological, probiotic, and technological attributes. The microbial profile of fermented toddy palm juice revealed a predominance of LAB over total viable bacteria and yeasts. Similar observations have been reported for other naturally fermented palm-based beverages, where LAB play a dominant role in fermentation by rapidly acidifying the substrate and suppressing competing microorganisms [5, 14]. The relatively lower yeast count observed in the present study suggests a supportive role in fermentation, likely contributing to flavor development rather than microbial dominance. The high LAB population highlights the suitability of toddy palm juice as a favorable environment for probiotic bacteria.

A total of ten LAB isolates were obtained and confirmed based on morphological and biochemical characteristics. The variation observed in acid and bile salt tolerance among these isolates underscores the strain-specific nature of probiotic properties, a phenomenon widely reported in earlier studies [4, 7]. Only three isolates (TPJ-L1, TPJ-L3, and TPJ-L7) demonstrated strong tolerance to both acidic pH and bile salts, indicating their potential to survive passage through the human gastrointestinal tract. Acid and bile tolerance are essential prerequisites for probiotics, as these conditions mimic the harsh environment of the stomach and small intestine [2].

Antimicrobial activity against foodborne pathogens is another important functional attribute of probiotic LAB. The selected isolates exhibited broad-spectrum antagonistic activity against both Gram-positive and

Gram-negative pathogens, including *Escherichia coli*, *Salmonella typhi*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and *Staphylococcus aureus*. The strong inhibitory effect observed against *S. aureus* and *B. cereus* is particularly significant, as these pathogens are commonly associated with food poisoning [15]. The antimicrobial action of LAB is generally attributed to the production of organic acids, hydrogen peroxide, and bacteriocin-like substances, which collectively inhibit pathogen growth [11]. Bile salt hydrolase (BSH) activity was detected in all selected isolates, indicating their ability to deconjugate bile salts and adapt to bile-rich environments. BSH activity is considered advantageous for probiotic survival and has been associated with cholesterol-lowering effects in the host [2]. The presence of BSH activity further supports the probiotic potential of the selected LAB strains. Safety evaluation is a critical aspect of probiotic selection. All selected isolates were non-hemolytic and DNase-negative, confirming their non-pathogenic nature. These findings are consistent with previous reports on LAB isolated from traditional fermented foods, which are generally regarded as safe when lacking virulence-associated traits [12, 3].

In addition to probiotic attributes, technological properties play a vital role in determining the applicability of LAB in food systems. The amylolytic activity observed in all selected isolates suggests their ability to hydrolyze starch into fermentable sugars, which is particularly beneficial for plant-based fermentations [13]. Among the isolates, TPJ-L7 exhibited higher amylase activity, indicating superior carbohydrate utilization efficiency. Exopolysaccharide (EPS) production was another notable technological feature of the selected LAB isolates. EPS-producing LAB are known to improve texture, viscosity, and mouthfeel of fermented products, while also contributing to product stability [1]. The strong EPS-producing ability of TPJ-L7 highlights its potential for use in functional beverage formulations. Additionally, EPS has been associated with health-promoting effects such as immunomodulation and prebiotic activity, further enhancing the functional value of these strains.

CONCLUSION

The present study highlights fermented toddy palm juice (*Borassus flabellifer*) as a valuable source of probiotic lactic acid bacteria with promising functional and technological attributes. A diverse microbial population dominated by LAB was observed, from which ten LAB isolates were obtained and screened for probiotic potential. Among them, three isolates (TPJ-L1, TPJ-L3, and TPJ-L7) demonstrated strong tolerance to acidic pH and bile salts, indicating their ability to survive gastrointestinal conditions. These isolates also exhibited broad-spectrum antimicrobial activity against common foodborne pathogens, supporting their role in enhancing food safety and gut health. The presence of bile salt hydrolase activity, along with the absence of hemolytic and DNase activities, confirmed the safety of the selected isolates. Furthermore, the observed amylolytic activity and exopolysaccharide production suggest their suitability for application in plant-based fermented beverages, contributing to improved fermentation efficiency, texture, and stability. Among the isolates, TPJ-L7 showed superior probiotic and technological performance. Overall, fermented toddy palm juice represents a promising non-dairy substrate for probiotic LAB, offering potential for the development of functional beverages and value-added traditional fermented products. Further in vivo studies and molecular identification are recommended to validate their health benefits and industrial applicability.

REFERENCES

1. Angelin, J., & Kavitha, M. (2020). Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules, 162*, 853–865. https://doi.org/10.1016/j.ijbiomac.2020.06.190
2. Begley, M., Hill, C., & Gahan, C. G. M. (2006). Bile salt hydrolase activity in probiotics. *Applied and Environmental Microbiology, 72*(3), 1729–1738. https://doi.org/10.1128/AEM.72.3.1729-1738.2006
3. Bhushan, B., Tomar, S. K., & Mandal, S. (2020). Probiotic attributes and safety assessment of lactic acid bacteria isolated from traditional fermented foods. *Food Bioscience, 35*, 100568. https://doi.org/10.1016/j.fbio.2020.100568
4. Charteris, W. P., Kelly, P. M., Morelli, L., & Collins, J. K. (1998). Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in the upper human gastrointestinal tract. *Journal of Applied Microbiology, 84*(5), 759–768. https://doi.org/10.1046/j.1365-2672.1998.00407.x
5. Corsetti, A., & Settanni, L. (2007). Lactobacilli in sourdough fermentation. *Food Research International, **40***(5), 539–558. https://doi.org/10.1016/j.foodres.2006.11.001
6. FAO/WHO. (2002). *Guidelines for the evaluation of probiotics in food*. Food and Agriculture Organization of the United Nations / World Health Organization.
7. Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., … 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. https://doi.org/10.1038/nrgastro.2014.66
8. Jansz, E. R., Wickramasekara, N., & Perera, C. O. (2015). Composition and properties of palm sap. *Journal of Food Composition and Analysis, 41*, 39–45. https://doi.org/10.1016/j.jfca.2015.01.005
9. Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, ***15***(2), 67–78. https://doi.org/10.1016/j.tifs.2003.09.004
10. Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., … Hutkins, R. (2017). Health benefits of fermented foods: Microbiota and beyond. *Current Opinion in Biotechnology, 44*, 94–102. https://doi.org/10.1016/j.copbio.2016.11.010
11. Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. *European Journal of Medicinal Chemistry*, *143*, 922–935. https://doi.org/10.1016/j.ejmech.2017.11.095
12. Sanders, M. E., Akkermans, L. M. A., Haller, D., Hammerman, C., Heimbach, J., Hörmannsperger, G., & Huys, G. (2010). Safety assessment of probiotics for human use. *Gut Microbes,* ***1***(3), 164–185. https://doi.org/10.4161/gmic.1.3.12127
13. Tsafrakidou, P., Michaelidou, A. M., & Biliaderis, C. G. (2020). Fermented cereal-based products: Nutritional aspects, possible impact on gut microbiota and health implications. ***Foods,*** ***9***(6), 734. https://doi.org/10.3390/foods9060734
14. Ukwuru, M. U., & Awah, J. I. (2013). Properties of palm wine yeast and its performance in ethanol production. *African Journal of Biotechnology*, ***12***(19), 2670–2677.
15. World Health Organization. (2015). WHO estimates of the global burden of foodborne diseases. WHO Press