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RESEARCH

Antibody-free Molecularly Imprinted Lateral Flow Platform For Rapid Lactic Acid Analysis in Fermented Foods

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Abstract

Rapid determination of lactic acid is critical for monitoring the quality and safety of fermented foods. However, existing analytical methods, including chromatographic, enzymatic, and antibody-based lateral flow assays, are limited by high cost, poor stability, or low suitability for small-molecule detection. In this study, we introduce **PASHA (Polymer-Assisted Selective Hybrid Analysis)**, a new analytical methodology that replaces biological receptors with molecularly imprinted polymers in lateral flow platforms Using PASHA, lactic acid was selectively recognized by imprinted poly(2-hydroxyethyl methacrylate) (pHEMA) nanoparticles and membranes integrated into a horizontal flow assay. Signal transduction was achieved via $FeCl_3$-mediated colorimetric complexation, enabling direct visual and semi-quantitative detection without antibodies or enzymes. The platform exhibited a linear response between 0.03 and 0.75% lactic acid ($R^2 = 0.99$). PASHA was validated using real fermented food samples including cheese, yogurt, kefir, ayran, and milk, showing strong agreement with conventional titration and spectrophotometric methods. Compared to classical immunochromatographic tests, PASHA offers superior chemical stability, lower cost, and enhanced performance for small organic acids. This work establishes PASHA as a new class of molecularly imprinted lateral flow methodology for food analysis, enabling robust, rapid, and field-deployable monitoring of fermentation and food quality.

Keywords Antibody-free Lateral flow test · Lactic acid imprinting · Antibody-free biosensor · Fermented foods

Introduction

Lactic acid is one of the most important metabolites in fermented foods, reflecting microbial activity, fermentation kinetics, and product quality. It plays a critical role in determining the taste, texture, shelf life, and microbiological safety of dairy products such as yogurt, cheese, kefir, and ayran, as well as fermented vegetables and beverages. In addition to food applications, lactic acid is also widely used in pharmaceuticals, cosmetics, biodegradable polymers, and biomedical materials (Upadhyaya et al. 2014; Alves de Oliveira et al. 2018). Accurate and rapid monitoring of lactic acid concentration is therefore essential for both food quality control and industrial process management.

Conventional analytical techniques used for lactic acid determination include high-performance liquid chromatography (HPLC), gas chromatography, enzymatic assays, titration, and spectrophotometry (Kang and Park 2016; Ayivi et al. 2020; Chen et al. 2023). Although these methods provide high sensitivity and accuracy, they require laboratory instrumentation, trained personnel, and long analysis times, which limit their suitability for real-time and on-site food monitoring. Although conventional analytical techniques such as HPLC, GC–MS, and spectrophotometric assays offer high sensitivity, their practical application is often limited by time-consuming sample preparation, high instrumentation

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costs, requirement for trained personnel, and reduced robustness when applied to complex food matrices. As the food industry increasingly demands rapid, decentralized, and cost-effective analytical tools, alternative sensing platforms are required.

Lateral flow assays (LFAs) are among the most widely used point-of-need analytical platforms due to their simplicity, portability, and visual readout (Wu et al. 2021; Tominaga and Ishii 2020; Zhang et al. 2020).

In food analysis, LFAs are commonly employed for the detection of pathogens, toxins, and allergens. However, classical LFAs rely on antibody–antigen recognition, which is fundamentally problematic for small molecules such as lactic acid. Small organic acids lack sufficient epitopes to enable sandwich-type immunoassays, forcing the use of competitive formats that suffer from low sensitivity, matrix effects, and poor robustness. Small-molecule analytes are generally not suitable for conventional lateral flow immunoassays because their single-epitope structure prevents the formation of a sandwich complex between capture and detection antibodies, resulting in insufficient signal generation (Ince and Sezgintürk 2022; Tominaga and Ishii 2020; Özyurt et al. 2022; İnce et al. 2023; Chen et al. 2024). Moreover, antibodies are expensive, temperature-sensitive, and chemically unstable in complex food matrices.

To overcome these limitations, molecularly imprinted polymers (MIPs) have emerged as synthetic recognition elements that mimic the selectivity of antibodies while offering superior chemical and mechanical stability. MIPs are formed by polymerizing functional monomers around a template molecule, generating binding cavities that are complementary in size, shape, and chemical functionality to the target analyte. Compared to antibodies, MIPs are inexpensive, reusable, resistant to harsh environments, and ideally suited for small-molecule recognition. Despite these advantages, the integration of MIPs into lateral flow platforms for food analysis has remained highly limited.

Here, we introduce **PASHA (Polymer-Assisted Selective Hybrid Analysis)**, a new analytical methodology that combines molecular imprinting with lateral flow technology to enable antibody-free detection of small organic molecules. In PASHA, molecularly imprinted polymeric membranes and nanoparticles serve simultaneously as the recognition element and signal-transducing interface. This eliminates the need for antibodies, enzymes, or biological labels and enables direct, robust detection in complex food matrices.

Using lactic acid as a model analyte, we demonstrate PASHA through a pHEMA-based molecularly imprinted lateral flow platform. Lactic acid binding is transduced into a visible colorimetric signal via $FeCl_3$ complexation, allowing rapid and semi-quantitative detection. The platform was validated using real fermented food samples including milk, yogurt, cheese, kefir, and ayran. By replacing biological receptors with synthetic molecular recognition, PASHA establishes a new class of lateral flow assays for food quality and fermentation monitoring.

Unlike conventional nitrocellulose-based lateral flow assays, the PASHA platform operates through capillary-driven horizontal transport across a porous, molecularly imprinted polymeric membrane. The lateral movement of liquid samples, subsequent interaction with immobilized recognition sites, and downstream signal development fulfill the fundamental operational principles of lateral flow assays, while enabling antibody-free detection of small organic molecules. Compared to conventional analytical platforms, PASHA introduces a simplified membrane-based design that enables rapid, low-cost, and equipment-free detection while maintaining acceptable analytical performance. Unlike instrument-dependent methods, PASHA is suitable for on-site analysis and demonstrates improved compatibility with complex food samples.

Unlike previously reported MIP-based lateral flow systems that rely on conventional strip architectures or external readers, PASHA integrates molecular imprinting directly into both the membrane and nanoparticle components, enabling antibody-free, enzyme-free, and equipment-free signal generation.

Experimental

Materials

Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), N- Isopropylacrylamide (NIPA), lactic acid, 2,2-Dimethoxy-2-phenylacetophenone (DMPA), co-monomer, potassium persulfate (KPS), ferric chloride ($FeCl_3$), methylene blue, and sodium hydroxide (NaOH) were purchased from Sigma Chem. Co. St. Louis, USA. In the study, two different polymeric materials were synthesized, one to be used on the surface and the other to be applied to the surface afterward. One of the synthesized polymeric materials was in nanoparticle form and was obtained through fluorescent labeling. The polymeric material used on the base was produced in membrane form. LA-imprinted HEMA nanoparticle = Polymer applied to the surface afterward Lactic Acid Imprinted Membrane = The polymeric surface onto which the sample is dropped. All solutions were adjusted to a target pH range of 5–7 to ensure reproducible complexation behavior and reaction stability.

Methods

Molecular Imprinting of Lactic Acid onto Polymeric Membrane

Molecular imprinting was carried out by mixing 2 mL of lactic acid with 4.37 ml of MBAA solution on a heated magnetic stirrer. This process facilitated the interaction

between lactic acid and MBAA, allowing the lactic acid-containing polymers to bind with MBAA (Scheme 1). Through this interaction, the formation of the pre-complex was completed.

Lactic acid and N-isopropylacrylamide (NIPA) were used at a defined molar ratio (1:1) selected to ensure sufficient functional group availability while preserving polymer network stability. The ratio was optimized based on preliminary experiments to balance sensitivity and membrane integrity. This mixture was homogeneously mixed in a container. The following additional materials were then added:

1845 µl distilled water
675 µl HEMA
75 µl pre-complex
30 µl EGDMA
12 mg DMPA

All of these mixtures were combined in a container and allowed to cool to facilitate (Scheme 2).

Although different monomer–template ratios were evaluated during preliminary trials, the 1:1 ratio was selected as it provided the best compromise between imprinting efficiency, membrane integrity, and reproducible lateral flow behavior.

Molecular Imprinting of Lactic Acid onto Polymeric Nanoparticles

Materials and Quantities Used for Pre-Complex Preparation:

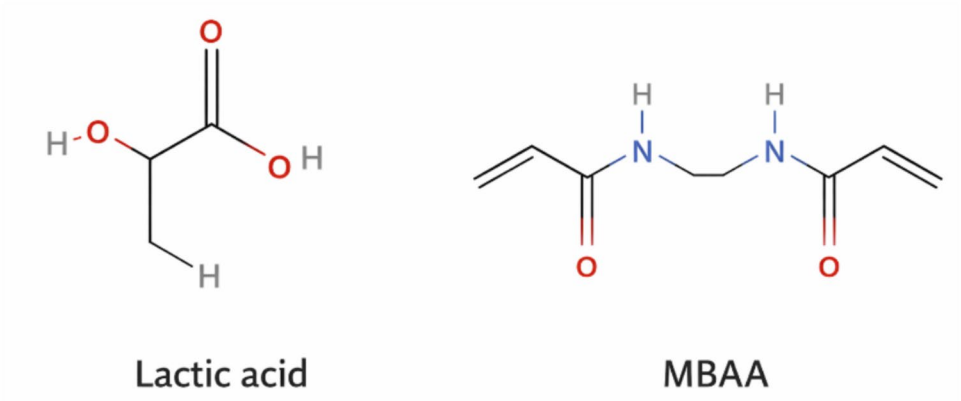
NIPA: 11.316 mg
Lactic Acid: 70.6 ml
Distilled Water: 10 ml
Molar ratio (1:1)

For the synthesis of HEMA nanoparticles, 0.275 g of PVA was dissolved in 25 ml of water. Then, a mixture of 0.6 ml HEMA and 0.3 ml EGDMA was added. The previously prepared pre-complex was then introduced into this mixture. Finally, 0.0198 g of KPS (potassium persulfate) and 45 ml of water were added to this mixture. KPS generated free radicals, initiating the polymerization reaction. Pre-complex (lactic acid + NIPA) + PVA + HEMA + EGDMA + IPA + KPS. After the mixture was prepared, nitrogen gas was introduced to remove oxygen from the mixture. The nitrogen gas ensured the removal of oxygen from the reaction environment, preventing oxygen from inhibiting free radical reactions and thereby increasing the efficiency of the polymerization process. In the final stage, the mixture was left in a water bath at 70 °C for 5 h to complete the synthesis, ensuring that the reactions were fully carried out.

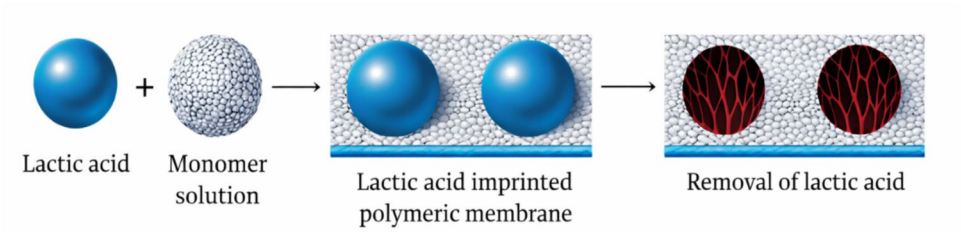
Washing Stage of LA Imprinted Membrane and Nanoparticles After polymerization, the lactic acid–imprinted cryogel-form membrane was gently immersed in distilled water and subsequently removed to eliminate unbound residues. Excess surface water was removed prior to further use.

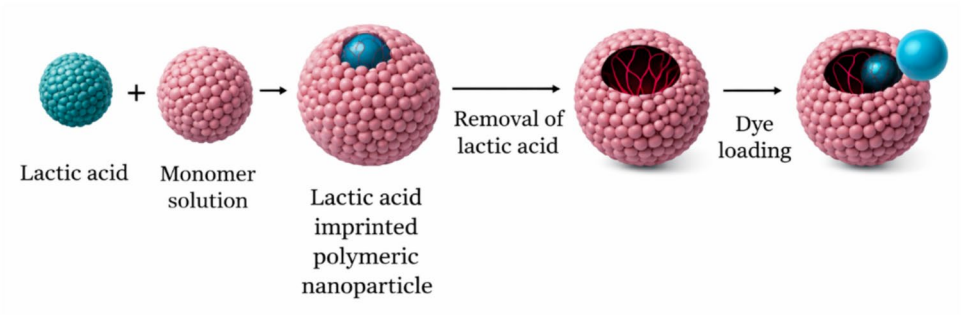
The LA-imprinted HEMA nanoparticle was centrifuged at 14,500 rpm for 20 min. After the centrifugation process, the first washing step was performed using alcohol. The

Scheme 1 Lactic acid + MBAA



Scheme 2 Preparation of lactic acid imprinted polymeric membrane





Scheme 3 Preparation of lactic acid imprinted and Methylene Blue labelled nanoparticles

second and third washes were conducted with distilled water. In the final washing stage, the mixture was stirred in an angled mixer. This process ensured that the mixture became homogeneous and effectively removed any remaining impurities. Finally, the mixture was centrifuged again to obtain the LA-imprinted HEMA nanoparticle, free of any chemical agents that did not participate in the polymerization.

Template Removal and Formation of Imprinting Sites Following polymerization, removal of the lactic acid template was carried out to generate specific recognition cavities within both the molecularly imprinted polymer (MIP) membranes and dye-loaded MIP nanoparticles. Due to the high water solubility and low molecular weight of lactic acid, ethanol was selected as the extraction solvent to facilitate efficient template removal without compromising polymer integrity.

Template elution was performed by repeatedly washing the polymeric materials with ethanol under gentle agitation. For both membranes and nanoparticles, multiple extraction cycles were applied, each consisting of solvent exposure followed by solvent replacement, to ensure complete removal of the template molecules. The washing procedure was repeated until no further lactic acid signal was detected in the elution solvent.

Successful removal of lactic acid and formation of imprinting sites were indirectly confirmed by spectrophotometric analysis using $FeCl_3$ as a complexation reagent. The absence of detectable $FeCl_3$–lactic acid complex formation in the final washing fractions indicated effective template removal. After template extraction, the imprinted polymers exhibited selective rebinding behavior toward lactic acid, confirming the availability of functional imprinting cavities.

Dye Loading onto LA Imprinted Nanoparticle 0,1 g of dry particles (Methylene Blue), 30 mg dye,10 ml distilled water, 0,4 g NaOH were kept in a water bath for 3 h. After the duration, the mixture was centrifuged, and excess dye was removed through washing steps (Scheme 3).

Under alkaline conditions, dye loading occurs predominantly through covalent attachment between reactive groups of the dye molecule and hydroxyl functionalities of the pHEMA-based polymeric network. The basic environment promotes nucleophilic activation of polymer hydroxyl groups, enabling stable covalent bonding rather than simple physical adsorption. This covalent immobilization mechanism contributes to enhanced signal stability and repeatability of the colorimetric response. A similar covalent dye–pHEMA interaction mechanism has been previously reported by Türkcan et al. for dye-functionalized poly(2-hydroxyethyl methacrylate) nanoparticles (Türkcan et al. 2014).

Characterization of Materials

SEM Scanning electron microscopy (SEM) was used to investigate the surface morphology of the polymeric membrane and the polymeric nanoparticles. Polymeric materials were dried for SEM examination. The surfaces were then coated with gold for 2 min to ensure conductivity. Samples were analyzed with a scanning electron microscope (Philips XL-30S FEG, the Netherlands). SEM imaging was performed at an accelerating voltage of 5–10 kV under high-vacuum conditions.

Zeta-Size Nanoparticleswere measured using a disposable cuvette from Zeta-size distribution (Nano ZS, Malvern Instruments, Malvern, UK) at 25 ºC. Three readings were taken for each sample.

Preparation of Solutions and Testing of Membranes

Lactic acid solutions were prepared at final concentrations of % range. Each concentration group was prepared by serial dilution from a stock solution under identical conditions to ensure consistency across experiments Samples were taken from the prepared membrane, and lactic acid (2 mL) was dripped onto the samples in varying concentrations:

%100 LA, %80 LA (1600 µl stock lactic acid + 400 µl d.water)

%50 LA (1000 µl stock lactic acid + 1000 µl d.water)
%25 LA (500 µl stock lactic acid + 1500 µl d.water), %0 LA

A total of 2000 µl of the dyed solution was dripped onto each membrane, and the results were observed. All experiments were performed in triplicate, and data are presented as mean ± standard deviation to assess repeatability.

Natural Product Applications

1 ml of kefir, milk, ayran and cheese samples were dropped onto each membrane, then 1 ml of dyed polymer was added to each membrane. The polymer was allowed to absorb into the membrane and excess polymer was removed from the surface. 1 ml samples taken from kefir, milk, ayran and cheese samples were dropped onto two different membranes. 1 ml of dyed polymer was added onto the membranes and the polymer was allowed to be absorbed by the membrane. When the excess polymer was removed from the surface, the highest coloration was observed in cheese. This was followed by ayran, kefir and milk samples.

Reference Analysis Kits

A mixture of 5% acid solution and distilled water was prepared and pinking reactions were investigated for the determination of lactic acid. The amounts of titrant applied to different samples were recorded and the results were compared with the coloration intensity.

Spectrophotometric Analysis

Cheese, yogurt, ayran, kefir and milk samples were determined spectrophotometrically in terms of lactic acid content. Each of the products was subjected to a 1000-fold dilution process and the analysis method was performed. The calibration chart was obtained using the same method with ($FeCl_3$) and the results of the samples were recorded.

PASHA Platform Assembly and Assay Workflow

In the PASHA platform, the molecularly imprinted polymer (MIP) membrane directly replaces the conventional nitrocellulose membrane used in classical lateral flow assays and serves as the stationary recognition surface of the system. Unlike antibody-based LFAs, no separate test or control lines are required. Upon application of a lactic acid–containing sample, lactic acid molecules migrate laterally and selectively bind to the imprinting cavities distributed throughout the MIP membrane. These binding sites immobilize lactic acid on the membrane surface, effectively localizing the analyte within the porous polymeric matrix. In the subsequent step, dye-loaded molecularly imprinted nanoparticles are introduced onto the membrane. These nanoparticles are themselves imprinted for lactic acid and carry physically entrapped dye molecules. When the nanoparticles encounter lactic acid molecules already bound to the membrane, specific imprinting interactions occur between the nanoparticles and the immobilized lactic acid. As a result of this interaction, the nanoparticles become anchored to the membrane surface at locations where lactic acid is present. This anchoring prevents further downstream movement of the nanoparticles in the direction of capillary flow. Consequently, dye molecules associated with the nanoparticles accumulate on the membrane surface, producing a visible color signal. The intensity of the observed coloration is directly related to the amount of lactic acid bound to the membrane, enabling semi-quantitative visual evaluation. In the absence of lactic acid, nanoparticles are not retained on the membrane and continue to migrate, resulting in minimal or no surface coloration (Scheme 4).

Results

In this study, the synthesis of lactic acid imprinted polymeric materials was successfully achieved using molecular imprinting and fluorescent labeling techniques.The results obtained demonstrate the usability of lactic acid-containing polymers, both in nanoparticle and membrane forms, for the detection of lactic acid in biological andindustrial applications.

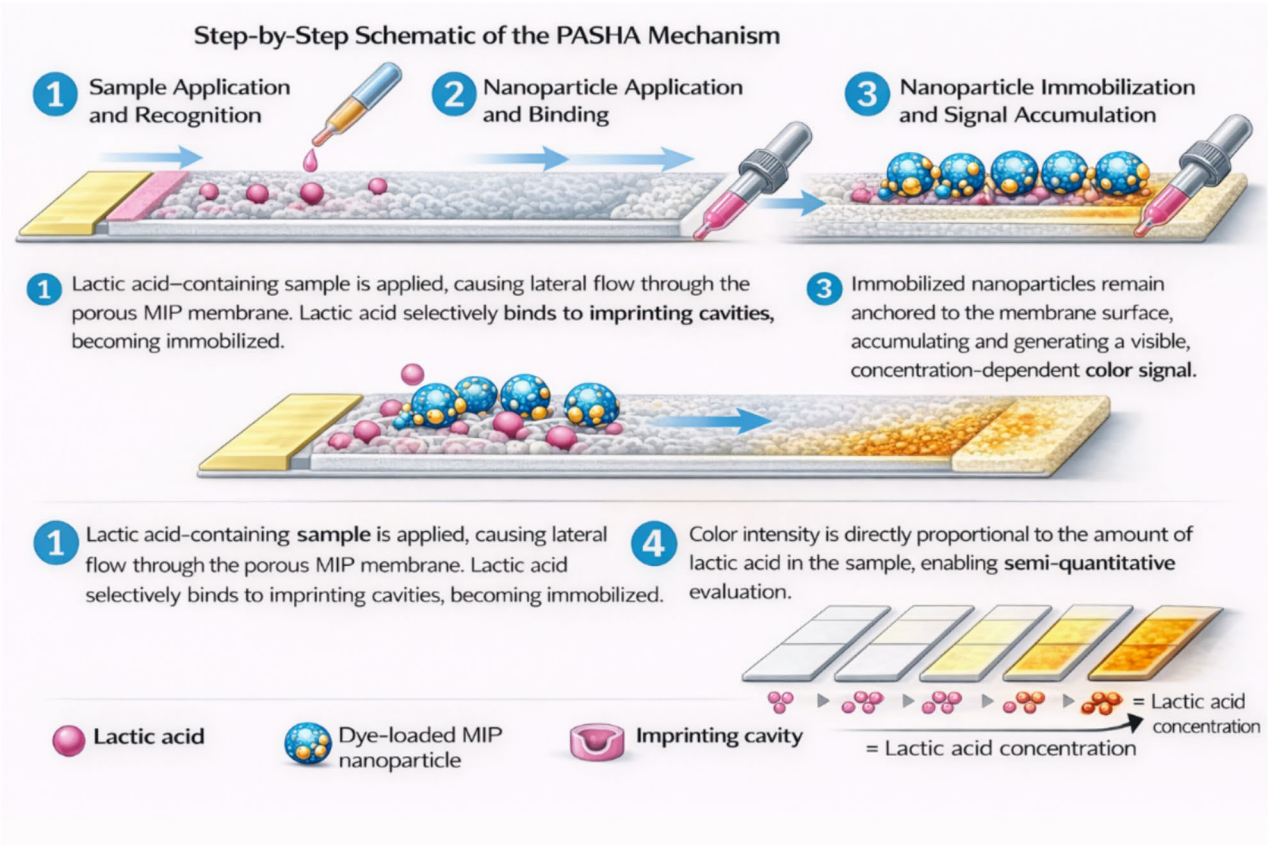
Spectrophotometer Measurements

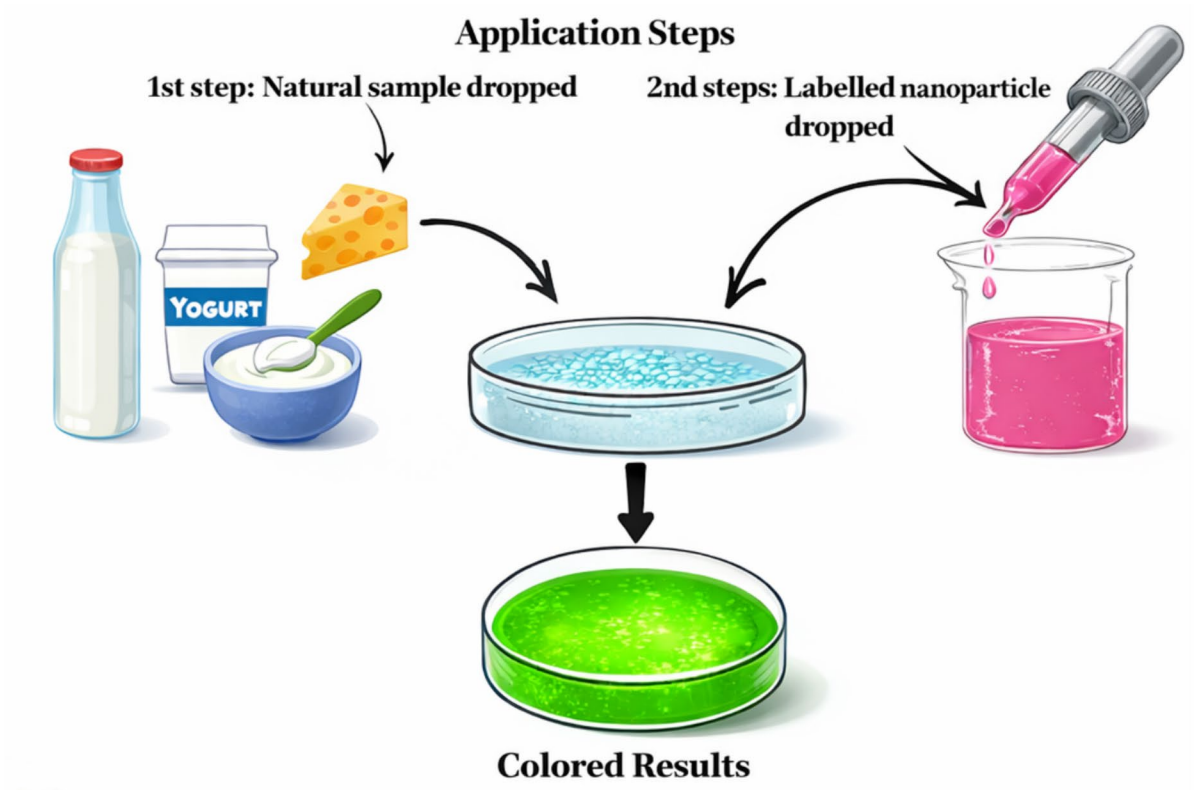
The specified concentration lines were prepared using lactic acid and distilled water mixtures, with the addition of FeCI3 (Fig. 1).

The obtained absorbance values indicate a linear relationship between the concentrations of lactic acid and FeCl3. The increase in values between concentrations demonstrates that the measurements are reliable and repeatable, confirming that the experiment was conducted accurately.

Polymer Synthesis and Characterization

Lactic acid-printed HEMA nanoparticles and membranes were synthesized using the molecular imprinting method, with lactic acid serving as the template. The polymerization strategy employed in this study is inspired by cryogel-based systems, which are traditionally used as column packing materials due to their high water retention capacity and excellent liquid permeability. However, unlike conventional cryogels synthesized as bulk monolithic structures for column-based applications, the present


Step-by-Step Schematic of the PASHA Mechanism
1 Sample Application and Recognition
2 Nanoparticle Application and Binding
3 Nanoparticle Immobilization and Signal Accumulation
1 Lactic acid–containing sample is applied, causing lateral flow through the porous MIP membrane. Lactic acid selectively binds to imprinting cavities, becoming immobilized.
3 Immobilized nanoparticles remain anchored to the membrane surface, accumulating and generating a visible, concentration-dependent color signal.
1 Lactic acid-containing sample is applied, causing lateral flow through the porous MIP membrane. Lactic acid selectively binds to imprinting cavities, becoming immobilized.
4 Color intensity is directly proportional to the amount of lactic acid in the sample, enabling semi-quantitative evaluation.
= Lactic acid concentration
= Lactic acid concentration
Lactic acid
Dye-loaded MIP nanoparticle
Imprinting cavity


Application Steps
1st step: Natural sample dropped
2nd steps: Labelled nanoparticle dropped
YOGURT
Colored Results

◂**Scheme 4** In PASHA, the imprinted polymeric membrane replaces nitrocellulose and acts as the analyte-capturing surface. Lactic acid binds to imprinting cavities on the membrane, after which dye-loaded imprinted nanoparticles selectively associate with the immobilized lactic acid. This interaction arrests nanoparticle migration, causing dye accumulation on the membrane surface and generating a visible, concentration-dependent color signal

approach adapts a cryogel-derived monomer system for the fabrication of a planar polymeric membrane synthesized under horizontal configuration. This design enables lateral liquid transport across the membrane, allowing its integration into a lateral flow–type analytical platform rather than a packed-column format. The nanoparticles were successfully labeled with fluorescence and observed under a microscope as yellow emissions. This indicates that lactic acid successfully bound to the polymer surface and that the labeling process was efficient. The removal of oxygen with nitrogen gas enhanced the efficiency of the polymerization (Fig. 2).

The effectiveness of the template removal process was further supported by the absence of residual lactic acid signal in blank control experiments and by the reproducible, concentration-dependent signal response observed during PASHA measurements.

The image depicts micropores and their respective dimensions. The membrane had porous features, however the nanoparticles were spherical and regular in size. SEM analysis revealed an average membrane pore size of approximately 24–32 µm, while nanoparticle size distribution analysis indicated a mean particle diameter of 500 nm with a narrow size distribution. ZETA size analysis indicated that the particle sizes were 280 nm. In addition, when the size distributions were analyzed, it was discovered that they were monomodal and had a uniform distribution (Fig. 3).

Dye Loading and Fluorescent Labeling

As a result of the dyeing process of lactic acid-containing polymers, the dye wassuccessfully integrated into the polymer structure. Membranes exposed to different concentrations of lactic acid were examined using fluorescent labeling, yielding positive results. 0, 0.25, 0.5, 0.8, 1 mg/mL Lactic acid concentrations were applied to the membrane surface and then lactic acid imprinted nanoparticle application was made. According to the results obtained, the color intensity increases as the concentration amount increases (Fig. 4).

Applications

The working logic of the kit is based on the neutralization of lactic acid. In the titration kit, which turns pink when it reaches a basic pH after becoming neutral, the dairy product containing the most lactic acid is cheese, while the product containing the least is milk.

The following results were obtained in the study conducted with the lactic acid determination kit:

- Cheese: Pinking was observed with 170 drops of lactic acid titrant.
- Yogurt: Pinking was observed with 115 drops of lactic acid titrant.
- Ayran: Pinking was observed with 55 drops of lactic acid titrant.
- Kefir: Pinking was observed with 80 drops of lactic acid titrant.
- Milk: Pinking was observed with 20 drops of lactic acid titrant (Fig. 5).

As a result of the analysis carried out using the lactic acid imprinted polymeric materials developed in the study, the product containing the highest amount of lactic acid was cheese, while the product containing the least amount of lactic acid was milk, in accordance with the titration test. It can be said that yogurt cannot be analyzed without pretreatment as a disadvantage compared to the other analysis method in the horizontal flow kit. Colorimetric responses were quantitatively analyzed by image-based intensity analysis under standardized lighting conditions to ensure consistency between visual readings. Each food sample measurement was performed in triplicate. Statistical significance was evaluated using appropriate statistical tests, and differences were considered significant at $p < 0.05$.

Spectrophotometric Analysis

According to the data obtained as a result of spectrophotometric analysis for lactic acid analysis, cheese (2%), yoghurt (1.5%), ayran (1%), kefir (0.8%) and milk (0.2%) were determined, respectively.

Lateral Flow Performance and Signal Reproducibility

The lateral flow behavior of the PASHA membrane was evaluated by monitoring capillary-driven transport of liquid samples across the horizontally synthesized porous polymeric membrane. Upon sample application, lateral migration toward the detection zone occurred within approximately 60–90 s, enabling rapid visual signal development without external pumping or instrumentation.

To evaluate the analytical acceptability of the PASHA platform, its semi-quantitative signal responses were directly compared with those obtained from the reference colorimetric method, which was considered as the true analytical

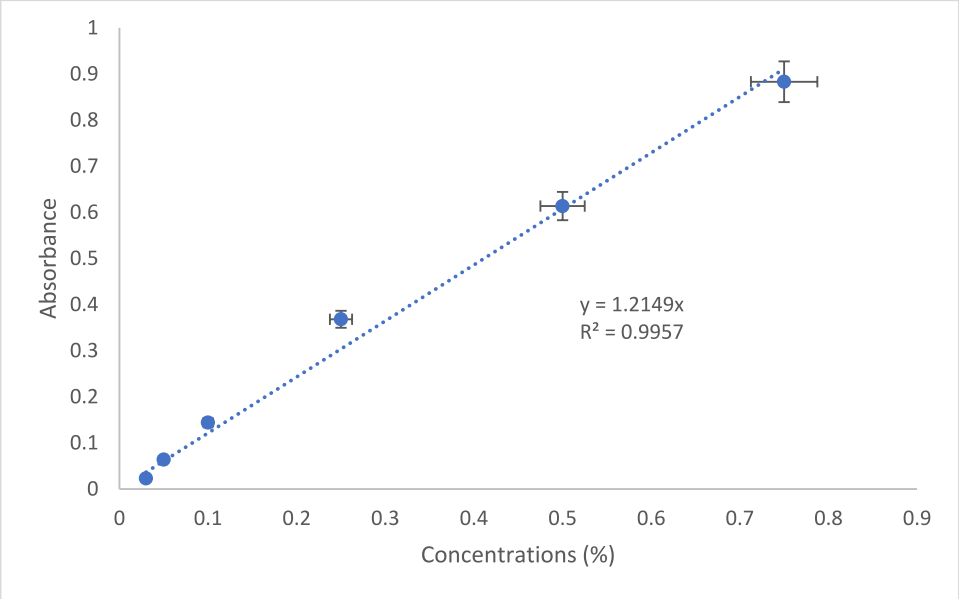


Fig. 1 Lactic acid calibration graph

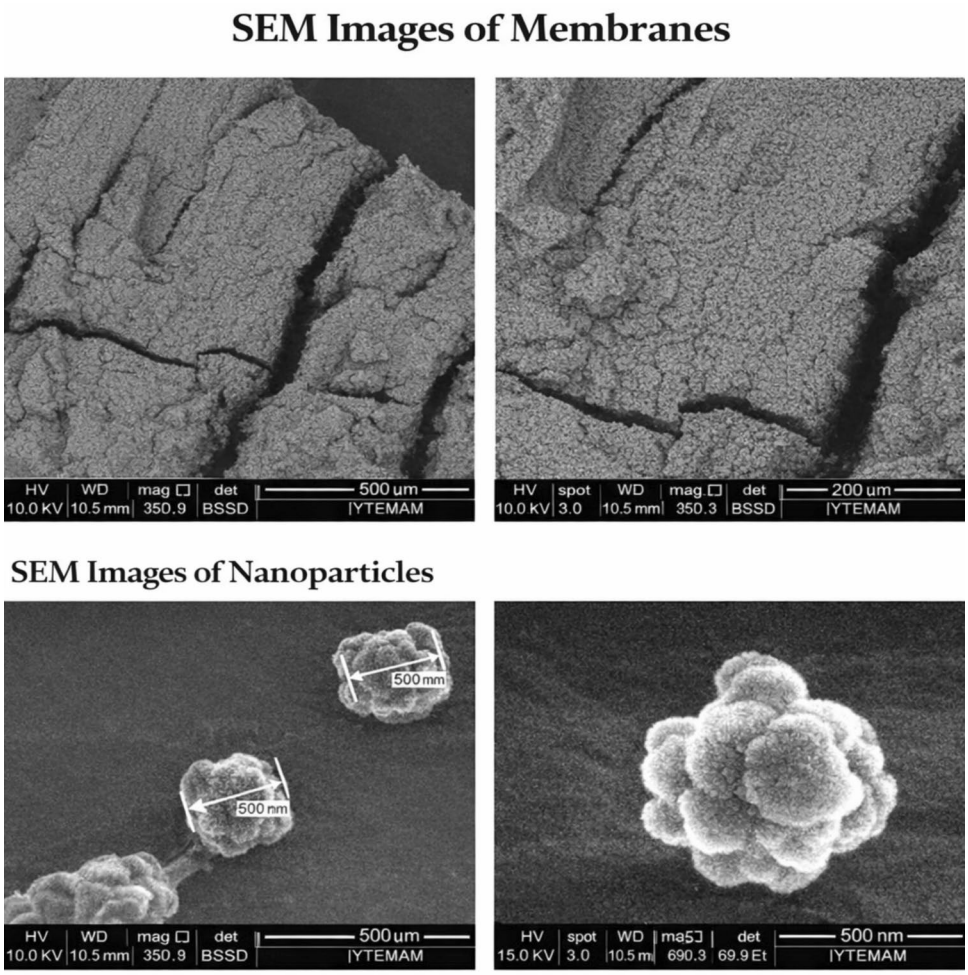


Fig. 2 Morphological structure of membrane and nanoparticles

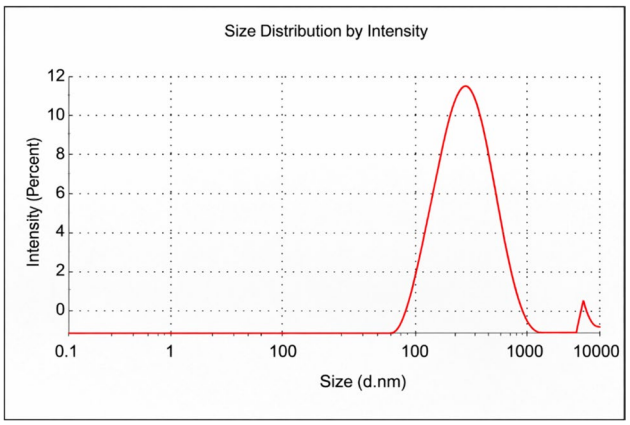


Fig. 3 Zeta-Size Analysis of LA Imprinted nanoparticles

response. For samples containing identical lactic acid levels, PASHA preserved the monotonic signal trend observed in the reference method (Fig. 6), with only minor deviations in normalized intensity values.

Quantitative comparison revealed that the relative error between PASHA and reference responses remained below 3% for all tested samples, while the root mean square error (RMSE) was calculated as 0.015. These results indicate strong agreement between the two methods and demonstrate that PASHA reliably reproduces concentration-dependent signal behavior despite its simplified, antibody-free lateral flow format.

Correlation Analysis

Correlation analysis between PASHA and the reference method further supported the analytical validity of the

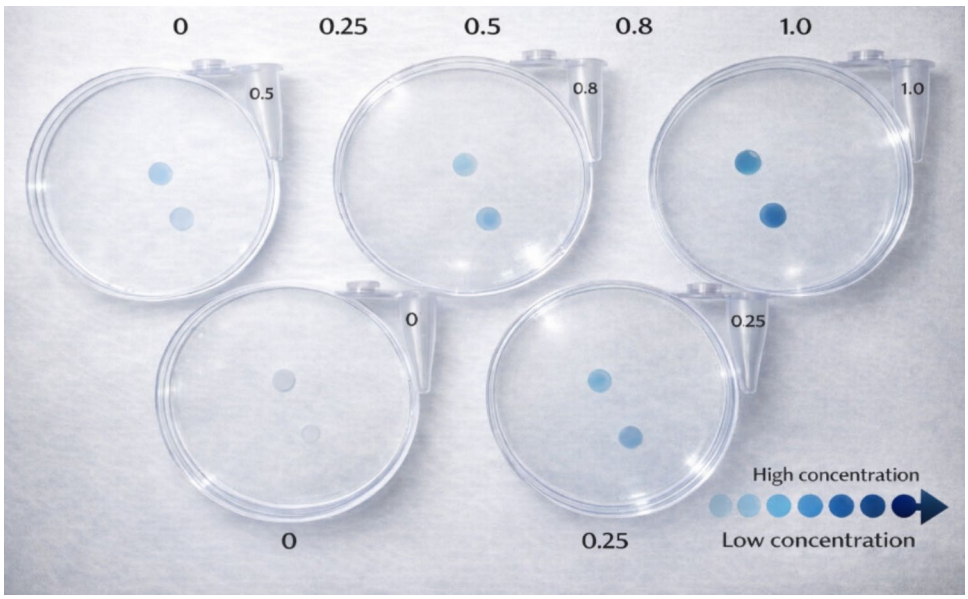


Fig. 4 Colorimetric response of the PASHA MIP-based membrane system at different lactic acid concentrations (0, 0.25, 0.5, 0.8, and 1.0). The gradual increase in color intensity corresponds to increasing lactic acid concentration, demonstrating a **semi-quantitative**, concentration-dependent visual response



Fig. 5 Determination of lactic acid in foods by kits (A: Reference kit, B: PASHA Method (Lateral Flow Test Kit, 1: Cheese, 2:Yogurt, 3:Ayran, 4:Kefir, 5:Milk)

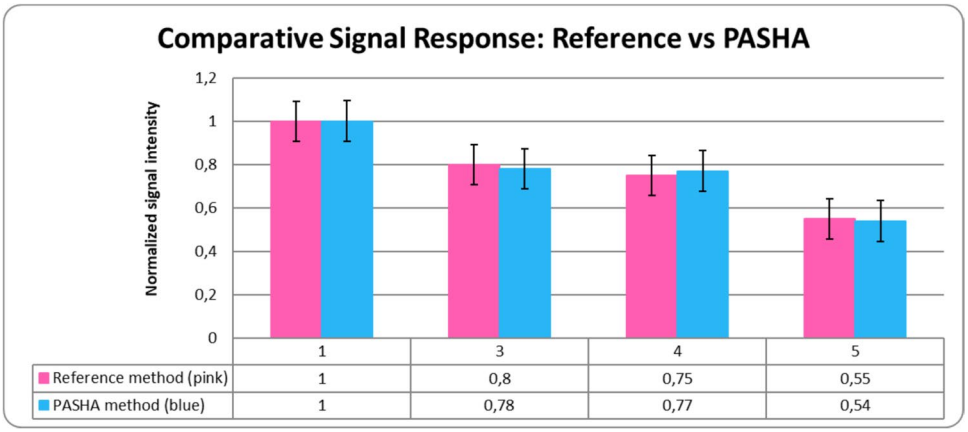


Fig. 6 Comparative signal response obtained using the reference colorimetric method (pink) and the PASHA-based lateral flow platform (blue) for samples containing identical lactic acid levels. Signal intensities were normalized to the highest response (Sample 1). PASHA exhibits a signal trend closely matching the reference method across all samples, demonstrating acceptable agreement and confirming its suitability for semi-quantitative lactic acid determination

platform. A linear relationship was observed between PASHA and reference signal intensities, indicating that PASHA accurately reflects changes in lactic acid concentration across the tested range. The high correlation coefficient confirms that PASHA can be considered analytically acceptable for semi-quantitative applications where rapid and field-deployable analysis is required.

Standard Deviation, Reproduction, and Acceptability

Signal reproducibility was evaluated by analyzing lactic acid standards in triplicate ($n = 3$). The calculated standard deviations were low across the tested concentration range, with relative standard deviation (RSD) values remaining below 8%. This level of variability is considered acceptable for semi-quantitative lateral flow assays and confirms the robustness of the PASHA platform under repeated measurements.

The analytical sensitivity of PASHA was estimated based on the image-based signal-to-noise characteristics of the calibration data. The limit of detection (LOD), defined as three times the standard deviation of the blank signal divided by the calibration slope, was estimated to be within the lower end of the tested concentration range. The limit of quantification (LOQ), calculated as ten times the standard deviation of the blank signal divided by the slope, fell well within the linear response region of the assay.

Although PASHA is not intended to replace laboratory-based quantitative techniques, the estimated LOD and LOQ values demonstrate that the platform provides sufficient sensitivity for practical monitoring of lactic acid levels in fermented food products.

Discussion

The findings of this study demonstrate the successful integration of lactic acid into polymeric structures, highlighting its suitability for analytical and sensing applications rather than solely structural or biomedical uses. The high solubility and flexibility of HEMA-based polymers enabled effective incorporation of lactic acid recognition sites, supporting their use in aqueous analytical environments.

In this work, a cryogel-inspired monomer system was adapted into a horizontally synthesized molecularly imprinted polymer (MIP) membrane, enabling lateral flow operation instead of conventional column-based formats. This approach bridges classical cryogel chemistry with lateral flow technology, extending the application scope of cryogel-derived monomers beyond traditional separation systems.

The developed PASHA-based platform provides a rapid, antibody-free, and visually interpretable detection strategy for lactic acid, offering a practical alternative to antibody-based lateral flow assays. The concentration-dependent colorimetric response observed on the MIP membrane allows semi-quantitative evaluation by the naked eye, which is particularly advantageous for on-site food analysis and quality control applications.

While the Results section presents the structural and analytical characteristics of the PASHA platform, a deeper interpretation of these findings is necessary to contextualize assay performance and limitations. In this regard, the morphological and physicochemical properties of the molecularly imprinted materials play a critical role in determining analytical behavior.

SEM analysis revealed a porous membrane architecture with interconnected voids, which is favorable for capillary-driven lateral flow and facilitates efficient transport of both

analyte molecules and nanoparticle probes. Increased membrane porosity enhances analyte accessibility to imprinting cavities, thereby improving binding efficiency and signal development. However, excessive porosity could potentially reduce mechanical stability and lead to non-uniform flow, highlighting the need for controlled membrane fabrication.

Zeta size (DLS) measurements demonstrated a narrow size distribution of the dye-loaded MIP nanoparticles, indicating good colloidal stability and size homogeneity. Such uniformity is essential for consistent interaction kinetics with membrane-bound lactic acid and contributes directly to signal reproducibility. Variations in nanoparticle size would be expected to affect surface binding probability and dye accumulation, thereby increasing signal variability.

The ability to perform rapid assays in different food matrices where lactic acid is commonly encountered may facilitate broader adoption of this diagnostic approach across the food industry. Future studies should focus on optimizing polymer composition, exploring alternative cross-linkers or co-monomers to improve mechanical stability, and expanding the system toward multiplex detection formats. Additionally, integrating advanced signal amplification strategies or digital readout systems may further enhance sensitivity and analytical performance.

Although the PASHA platform shows preferential recognition toward lactic acid, partial cross-reactivity with other hydroxy-containing organic acids cannot be completely excluded. Therefore, PASHA is positioned as a semi-quantitative screening tool rather than a fully selective quantitative assay. Although long-term stability testing was not performed in this study, the inherent chemical robustness of molecularly imprinted polymers suggests superior shelf-life compared to antibody-based systems. This will be addressed in future work.

The performance of PASHA in real food matrices is influenced by the physicochemical complexity of fermented products. Proteins, lipids, and coexisting organic acids may compete with lactic acid for access to imprinting sites or alter flow behavior within the membrane matrix. These matrix effects were particularly evident in yogurt samples, where elevated protein content and higher viscosity impeded capillary flow and reduced effective analyte diffusion.

In contrast, liquid matrices such as milk and fermented beverages exhibited more consistent signal development, supporting the suitability of PASHA for low-viscosity food systems. These observations underscore the importance of matrix-dependent optimization and justify the classification of PASHA as a screening-level analytical tool rather than a universal quantitative method.

The colorimetric readout employed in PASHA offers several practical advantages, including operational simplicity, enzyme-free signal generation, and rapid visual interpretation. These features make the platform particularly attractive for on-site and resource-limited settings.

Nevertheless, the $FeCl_3$-based detection chemistry is inherently sensitive to hydroxy-containing organic acids, which may limit absolute selectivity under certain conditions. For this reason, PASHA is not intended to provide absolute quantification but rather a semi-quantitative assessment of lactic acid levels. When combined with reference methods, the platform can serve as an effective preliminary screening tool to identify samples requiring further laboratory analysis.

Overall, the presented validation results demonstrate that PASHA meets the fundamental analytical performance criteria required for a semi-quantitative lateral flow assay. When compared with conventional analytical methods reported in the literature, PASHA demonstrated comparable performance in food samples while offering advantages in speed, simplicity, and cost. The platform exhibits acceptable reproducibility, low relative error compared to reference methods, and strong correlation in signal response. These characteristics, combined with its antibody-free design and rapid assay time, support the analytical acceptability of PASHA for on-site and screening-level lactic acid analysis in fermented foods.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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