

RESEARCH ARTICLE

Development of highly sensitive cuvette-type localized surface plasmon resonance (LSPR) sensing chips for the rapid detection of tyramine in spiked samples of beef, mackerel, and cheese

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Abstract

The globalization of food distribution has made it necessary to ensure the delivery of fresh food to the consumers. Protein-rich foods produce a significant amount of tyramine when spoiled. Therefore, it was envisaged to develop localized surface plasmon resonance (LSPR)—sensor for monitoring tyramine content in beef, mackerel, and cheese. For this purpose, the sensors were developed by coating the LSPR chip with gold nanoparticles (AuNPs) followed by conjugation of tyramine-specific peptide (TYR1). The TYR1 was synthesized and screened by phage display and binding efficiency was evaluated by DockThor. The optimized LSPR sensor showed highest binding efficiency at TYR1 concentration of 20 µg/mL with reaction time of 40 min. The sensor also showed an excellent detection range for beef, mackerel, and cheese were 0.01–10 ppb, 0.01–10 ppm, and 0.01–10 ppb, respectively, in comparison to standard. These findings indicated that this sensor can be used as a promising tool for on-site monitoring of food quality.

KEYWORDS

gold nanoparticle, LSPR, on-site detection, peptide, peptide screening, phage display, tyramine

1 | INTRODUCTION

Population and economic growth throughout the world have greatly increased the demand for food products (Baker & Friel, 2016). Protein-rich food consumption has risen over the past few decades and is expected to keep rising (Wood, 2023; Xu et al., 2021). Thus, the producers have been forced to store it in such a way that it could not

degrade. Although, the spoilage of such foods cannot be avoided due to the exposure of environmental factors (Sannigrahi et al., 2023). The degradation of protein-rich foods results in the production of several biogenic amines (BAs). BAs are a low molecular weight nitrogenous organic compound that is produced by the decarboxylation of certain amino acids in food by microorganisms and making it a good indicator of food spoilage and freshness (Gao et al., 2023; Sannigrahi et al., 2023). High quantity of BAs in conventional foods is highly toxic to human health when consumed. The consumption of spoiled food products poses multiple health hazards, including carcinogenic

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effects, endocrine malfunction, headache, hypercoagulability, gastritis, ulcers, and allergic reactions. The European Food Safety Authority (EFSA) recommends a tyramine intake limit of 600 mg in a single meal for healthy people. In particular, patients taking monoamine oxidase enzyme inhibitors such as antidepressants may experience severe hypertension, vomiting, and cardiovascular disease (Dala-Paula et al., 2023). The onsite or real-time detection of tyramine may give us the opportunity to avoid the distribution of spoiled foods to the consumers. Existing techniques for the detection of tyramine in food are time consuming, require expertise and expensive equipment. Therefore, there is a need for sensor technologies that enable simple and rapid point-of-care monitoring of tyramine in food.

Biosensor utilizes a biologically recognized functionalized substrate to detect a target molecule (Deka et al., 2022; Sannigrahi et al., 2023; Sepúlveda et al., 2009; Shin et al., 2023). Biosensors have been widely employed in various fields such as food safety, medical diagnosis, and environmental monitoring. A large volume of past research has focused on improving the specificity, affinity, and selectivity of biosensors for target biomolecules in order to develop portable sensors (Cho et al., 2022; Heo & Ha, 2022; Park et al., 2022; Son et al., 2023). Biosensors based on localized surface plasmon resonance (LSPR) are ideal for miniaturization, rapid, and easy detection. LSPR is a collective oscillation phenomenon caused by interactions between light and electrons of, for example, gold nanoparticles (AuNPs) at an interface between a metal surface and a dielectric (Anker et al., 2008), and this phenomenon allows surface states to be monitored and molecular interactions to be analyzed (Li et al., 2018). LSPR measures changes in the refractive index induced by LSPR from a maximum absorption wavelength following exposure of metal nanoparticles to ultraviolet (UV)-visible spectroscopy (Zook et al., 2011). The LSPR units have higher sensitivity, and the size of the detection unit is 100 times smaller than that of surface plasmon resonance (SPR) spectroscopy. In addition, the LSPR utilizes less expensive unpolarized UV-visible spectroscopy (Kim et al., 2009). Therefore, LSPR-based biosensors have the potential to overcome the detection limit of SPR and reduce manufacturing costs (Gabudean et al., 2011). LSPR sensors based on novel metal nanoparticles have proven to be an effective platform for the detection of biological targets (Anker et al., 2008; Baciú et al., 2008). The researchers have attempted to synthesize effective plasmonic particles by using various metals such as Au, Ag, and HgS (Chen et al., 2015; Jia et al., 2013), and also they have successively manipulated the shape of nanoparticles such as nanoplates, nanorods, mushrooms, and nanoprisms in order to enhance plasmonic signals (Sanders et al., 2014; Shen et al., 2013; Zhang et al., 2016). Others have reported the development of LSPR sensors that can utilize various receptor types, such as aptamers, peptides, antibodies, and chemicals, in order to optimize the selective binding with the target molecule (Lee et al., 2018; Li et al., 2016; Zhang et al., 2014).

Despite past research on plasmonic nanoparticle synthesis and specific receptor screening, there have been few reports on the optimal methodology for the deposition of plasmonic nanoparticles and the fabrication of portable substrates that can provide stable and reproducible plasmonic signals (Coskun et al., 2014; Galush et al., 2009; Hsu et al., 2011; Im et al., 2014). Methods of screening peptides that selec-

tively respond to a specific substance represent another challenge, which has been resolved by the phage display technique (Cho et al., 2023). In this technique, the phage displays the expressed peptides on its surface, followed by the development of a specific peptide that binds strongly to a particular target (Bang et al., 2021; Shin et al., 2023; Smith & Petrenko, 1997). It has the advantage of amplifying phages with the desired peptide (Pande et al., 2010). This technique has been widely used in the study of selecting functional peptides that bind to a specific target (Shin et al., 2023).

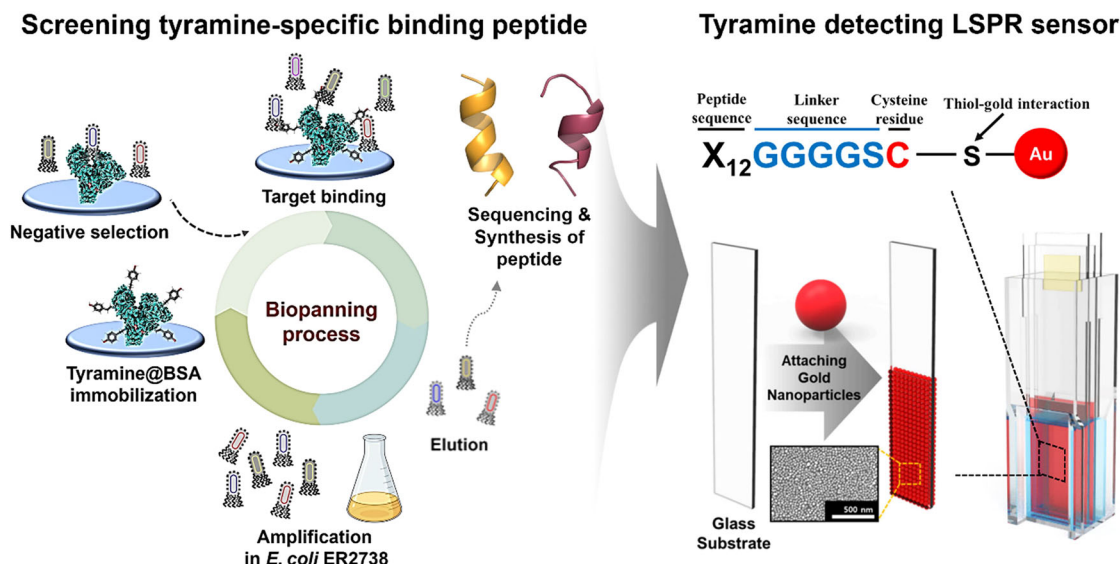
The plasmonic nanoparticle-based LSPR biosensors have attracted the attention of many scientists in biotechnological and clinical chemistry because of their high sensitivity, low cost, reliability, reproducibility, and rapid and selective recognition of BA (Yoo et al., 2015). Recently, various types of LSPR sensors have been established and used for the detection of environmentally toxic heavy ions, protein toxins, carbohydrates, caseins, nucleic acids, and biomolecules (Bellapadrona et al., 2012; Cheng et al., 2014; Jayabal et al., 2015; Yeom et al., 2013). However, many of these LSPR biosensors have low reproducibility, and it is difficult to immobilize large areas of plasmonic-active nanoparticles while reducing the detection limit. Despite the progress in new approaches developed for the simple and rapid recognition of tyramine, the research to improve these shortcomings still needs to be further delineated. However, the combination of AuNPs and peptide in the LSPR-based detection of tyramine has not yet been developed. It has also been reported that LSPR-based biosensors have the advantages of being fast, highly sensitive, label-free, and simple to set up.

Therefore, the present study was focused to design and develop an LSPR sensor for the onsite and fast detection of tyramine by LSPR chip coated with AuNP and linked with a screened tyramine-specific peptide. Finally, the aim was to contribute to the field of food and beverage industry by presenting a highly sensitive LSPR sensor that can quickly detect tyramine in the field without complicated processes during the distribution process or before consumption. The peptides that reacted selectively to tyramine were screened using a phage display technique using M13 phages. The tyramine-specific peptides were screened through phage display technology. The LSPR sensor with high sensitivity has been fabricated and employed in the present study by screening peptides that selectively bind to tyramine, as shown in Scheme 1.

2 | MATERIAL AND METHODS

2.1 | Reagents and materials

Ph.D.TM-12 phage display peptide library kit, which contain Ph.D.TM-12 peptide library, -96 gIII sequencing primer, biotin, and *Escherichia coli* ER2738, was purchased from New England Biolabs. Bacto-tryptone, yeast extract, sodium chloride, and agar powder were obtained from Duchefa Biochemie. 4-Morpholineethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxy succinimide (NHS), potassium hydroxide, glutaraldehyde (GA), gold (III) chloride trihydrate (HAuCl₄·3H₂O), tyramine, (3-aminopropyl)



SCHEME 1 Schematic diagram illustrating the screening and conjugation of peptides (TYR1 and TYR2) and the fabrication of tyramine detecting localized surface plasmon resonance (LSPR) sensor. In the LSPR biosensor, binding of TYR 1 peptide to the gold nanoparticles (AuNPs)-coated chip for detecting tyramine.

triethoxysilane (APTES), bovine serum albumin (BSA), sodium bicarbonate, trisodium citrate dihydrate, tyramine receptor, cadaverine, histamine, ammonia, ethanol, and amicon ultra-0.5 mL 3K were obtained from Sigma-Aldrich Chemical Co. 1-Step™ Tetramethylbenzidine (TMB)-Enzyme-linked immunosorbent assay (ELISA) substrate solution was purchased from Thermo Scientific™. Beef, mackerel, and cheddar cheese were procured from the local market. All the chemicals used were analytical grade of purity. The 96-well microplates were obtained from Costar Inc.

2.2 | Preparation and characterization of tyramine and BSA conjugates

The synthesis of tyramine conjugated with BSA (tyramine@BSA) involved the coupling the amino group of tyramine to carboxyl group of BSA in the presence of EDC/NHS, following a previously reported procedure (Cho et al., 2023). Briefly, 0.3 mg of tyramine was dissolved in 300 μ L of MES buffer (pH 6.0). Separately, 2 mg of BSA was dissolved in 200 μ L of MES buffer. The tyramine and BSA solutions were mixed with 10 mg of EDC and 4 mg of NHS followed by the incubation of reaction mixture for 2 h at 25°C to facilitate the coupling reaction. The tyramine@BSA conjugates were purified by centrifugation at 10,000 $\times g$ for 20 min using 0.5 mL Amicon tube, followed by washing with deionized (DI) water.

The tyramine@BSA conjugates were characterized and verified by using the AB SCIEX matrix assisted laser desorption ionization (MALDI)-time of flight (TOF)/time of flight tandem mass spectrometry (TOFTM) 5800 system (Applied Biosystems) at the Korean Basic Science Institute (KBSI). This analytical approach allowed for precise and reliable characterization of the synthesized tyramine@BSA conjugates. Both the BSA and tyramine@BSA samples were prefiltered through a 0.22 μ m syringe filter to ensure purity and remove any particulate mat-

ter. In brief, a sinapinic acid matrix saturated in 30% (v/v) acetonitrile (ACN) in DI water containing 0.1% (v/v) trifluoroacetic acid (TFA) was used for the MALDI-TOF/TOFTM analysis.

2.3 | Screening of tyramine-specific peptide sequences by phage display technique

The screening of tyramine-specific peptides was performed by the phage display technique which includes biopanning. The biopanning process employed the M13 bacteriophage library aimed to identify the tyramine-specific peptides, as outlined in previous study (Kim et al., 2023). In brief, prior to biopanning, the tyramine@BSA-coated plate was prepared by treating a polystyrene (PS) plate with 1% KOH for 10 min, followed by 2% APTES for 1 h at 80°C to functionalize amino group on PS plate surface. After that, 1% GA in 0.05 M carbonate buffer (pH 9.6) was added and incubated for 2 h at 37°C followed by the immobilization of tyramine@BSA (10 μ L) dissolved in 0.1 M NaHCO₃ for overnight at 4°C. This preparation ensured the availability of the target molecules on the plate surface.

To initiate the biopanning process, 1% BSA solution in DI water was added into the tyramine@BSA-coated plate for 1 h at 37°C followed by six times washing with 0.5% phosphate-buffered saline (PBS) containing Tween-20 (0.5% PBST, pH 7.4). The dodecapeptide from display library (1×10^{11} PFU/mL) was added to the surface of the tyramine@BSA-coated plate and incubated it for 1 h at room temperature (RT) with gentle shaking and washing for 10 times with 0.5% PBST, ensuring the removal of any unbound phage particles. The tyramine specifically bound phage particles were then eluted using 0.2 M glycine-HCl buffer (pH 2.2) with 1 mg/mL BSA and neutralized by 1 M Tris-HCl (pH 9.1). The eluted phage particles were amplified by infecting *E. coli* ER2738. The infection was conducted by incubating with early-log phase *E. coli* cultures for 5 h with shaking at 37°C. The

amplified M13 phage particles were purified by adding 1/6 volume of the 20% polyethylene glycol (PEG) 8000/2.5 M NaCl followed by centrifugation at $12,000 \times g$ for 10 min. The phage candidates from the eluate were sequenced by SolGent to investigate the sequence of tyramine-specific bound phage particles.

2.4 | Determination of binding affinity of phages for tyramine using ELISA

To assess the binding affinity of phage candidates for tyramine, BSA-coated ELISA plate was considered as a control. In brief, the wells of protein (BSA and tyramine@BSA)-coated ELISA plates were blocked with a 0.5% BSA solution for 30 min at 37°C followed by washing with 0.5% PBST. After that, the phage particles ($100 \mu\text{L}$ of 5×10^{11} PFU/mL) were added into the wells and incubated for 1 h at 25°C, allowing them to interact with the BSA and tyramine@BSA followed by washing with 0.5% PBST to remove any unbound phages. To detect the specific binding of phages, M13-horseradish peroxidase (HRP) antibody ($100 \mu\text{L}$ in 0.5% BSA solution) was added to each well and incubated for 1 h at 25°C followed by washing with 0.5% PBST. TMB ($100 \mu\text{L}$) was added to the wells and incubated for 30 min at 25°C to develop the color. The reaction was stopped by adding stopping buffer (2 M H_2SO_4), and the absorbance was measured by using a microplate reader (Multiskan GO, Thermo Scientific) at 450 nm.

2.5 | Molecular docking simulation with DockThor

For conducting peptide-tyramine docking studies, we utilized the freely available DockThor web server (Santos et al., 2020). To facilitate the docking process, we first predicted the structure of the TYR1 peptide using Pep-Fold3.0, while obtaining the tyramine structure from PubChem (PubChem CID: 5610). The DockThor algorithm parameters were set to 24 docking runs, 1,000,000 evaluations per docking run, with a population of 750 individuals. By defining the search space using a grid box, the DockThor program efficiently identified top energy docking poses ranked by the total energy (E_{total}), which were selected for subsequent virtual screening experiments and binding affinity predictions. The selection of models was chosen based on the lowest average root-mean-square deviation (RMSD), ensuring suitability for docking study.

2.6 | Synthesis of gold nanoparticles (AuNPs)

AuNPs were synthesized by Turkevich/Frens reaction (Yoo et al., 2022). In a three-neck flask installed with water condenser and heating mantle, 200 mL of distilled water (DW) was added with 2 mL of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ solution (40 mg/mL) and heated up to 380°C at 200 rpm. When the solution started to boil, trisodium citrate solution (228 mg in 20 mL of DW) was added. After 5 min, the reaction mixture turns into red-wine color, which confirms the presence of synthesized

AuNPs. The resulting AuNPs were collected by centrifuging the reaction mixture at 1000 rpm for 30 min and washed with DI water. The resulting AuNPs were characterized by using the particle size analyzer (ELS-Z, Otsuka Electronics), transmission electron microscopy (TEM) (CM200, Philips), and scanning electron microscopy (SEM) (SU8010, HITACHI).

2.7 | Fabrication of cuvette-form LSPR sensor

Commercially available tempered glass LSPR biosensor chip was used as a backbone for preparing the tyramine-specific LSPR biosensor by previously established methods with slight modifications (Yoo et al., 2022). The procured LSPR sensor chips (8 mm $\times$ 50 mm) were immersed in methanol (35 mL) and sonicated for 20 min followed by washing in DI water. The glass biosensor chips were immersed in 0.5% APTES and incubated in oven at 60°C for 1 h followed by washing with DI water. After that, these APTES functionalized glass biosensor chips/substrates were immersed in a cuvette containing previously synthesized 800 μL of AuNPs and incubated for overnight following the washing with DI water. The two AuNP-coated glass substrates and three plastic chips were combined with double-sided tape, and it was immersed in a cuvette filled with DI water. That glass chip was analyzed by SEM (SU8010, HITACHI) to confirm the even distribution of AuNPs on the glass surface.

2.8 | Immobilization of antityramine peptide on AuNPs-coated glass chip surface

The antityramine peptides were immobilized onto the AuNP-coated glass chips. The immobilization was done using a previously described method (Yoo et al., 2022) with slight modifications. In brief, the AuNP-coated glass chips were immersed in cuvette containing 800 μL of peptide solution (20 $\mu\text{g}/\text{mL}$) and incubated for 40 min followed by washing with DI water. After washing, these glass chips were immersed in a cuvette containing 800 μL of 1% BSA. After 20 min of reaction period these glass chips were washed with DI water. To confirm the immobilization of antityramine peptide on the surface of the AuNPs-coated glass chips, the change in absorbance according to the concentration of TYR1 was calculated by subtracting the maximum absorbance of the AuNPs-coated glass chip from that of the antityramine peptide-immobilized glass chip. The absorbance of the biosensor chip was scanned from 400 nm to 700 nm using an UV-visible spectrometer (Jasco V770).

2.9 | Assay for the selective detection of tyramine using cuvette-form LSPR sensor

The tyramine detection performance of LSPR biosensor was fabricated and used for evaluating the standard tyramine solution. First, standard tyramine samples with a different concentration ranging from 0.01 ppb

to 10 ppm were prepared to measure its sensitivity. The LSPR biosensor was immersed in a cuvette containing 800 μ L of standard tyramine samples and incubated for 20 min. After the incubation period, this LSPR sensor was washed with DI water followed by the UV-visible spectrophotometric analysis (absorbance spectra 400–700 nm) by scanning the LSPR biosensor to confirm the detection of tyramine.

In other sets of experiments, different solutions of cadaverine, histamine, methanol, ammonia, and ethanol (10 ppm each) were also subjected to expos against our newly developed biosensor to analyze the selective and comparative sensing. In brief, the LSPR sensors were immersed in cuvettes containing 800 μ L of different solutions of cadaverine, histamine, ammonia methanol, and ethanol and incubated for 20 min followed by washing with DI water. After washing, the sensors were scanned against the absorbance spectra of 400–700 nm to confirm the selective detection of tyramine by our newly developed biosensor. The absorbance of the biosensor chip was scanned from 400 nm to 700 nm using an UV-visible spectrometer (Jasco V770).

2.10 | Detection of tyramine in spiked samples of beef, mackerel, and cheddar cheese using cuvette-form LSPR biosensor

Based on the detection of the standard tyramine, the newly developed LSPR biosensor was subjected to quantitatively detect the tyramine in spiked samples of beef, mackerel, and cheddar cheese. Beef, mackerel, and cheddar cheese were purchased from local grocery stores and processed for the experimental procedures. In brief, 0.5 g of each fresh food samples were cleaned and chopped. The 2.5 g of silica beads (1, 3, and 5 mm) followed by the 12 mL of dithiomethyl sulfoxide (DMSO) were added into 15 mL polypropylene vial separately. The different size of silica beads was used to facilitate the complete lysis of beef, mackerel, and cheddar cheese samples. The mixture was vortexed and filtered to separate the liquid part. The addition of 12 mL DMSO and filtration was repeated one more time and the filtrate was used for tyramine spiking. The tyramine-spiked samples were dipped in LSPR sensor and scanned against the 400 nm to 700 nm using an UV-visible spectrometer (Jasco V770) to calculate the limit of detection (LOD). The LOD value was calculated by following the previously described method (Oh et al., 2019; Robouch et al., 2016).

$$\text{LOD} = 3.9 \times \frac{\text{Standard deviation of blank}}{\text{slope of calibration curve}}$$

2.11 | Quantitative detection of tyramine by high-performance liquid chromatography (HPLC)

The quantitative detection of tyramine in the standard tyramine solution and spiked food samples supernatant were performed by previously described method with slight modifications (Molins-Legua et al., 1999). In brief, for the derivatization of standard tyramine and the homogenized beef, mackerel, and cheese samples, 1 mL of standard

tyramine and the supernatant of homogenized samples was mixed in their respective tubes with 200 μ L of 2 M sodium hydroxide, 300 μ L of saturated sodium bicarbonate and 2 mL of a dansyl chloride solution (10 mg/mL) prepared in acetone were added to the mixture, which was then incubated at 40°C for 45 min. The residual dansyl chloride was removed by adding 100 μ L of 25% ammonium hydroxide and incubated for 30 min at 25°C. After incubation period, the total volume of reaction mixture was adjusted to 5 mL with ACN. The total reaction mixture was centrifuges at 3000 rpm for 5 min and filtered by 0.2 μ m-pore-size filters. The supernatant was collected for the tyramine detection by HPLC using the UV detector at 254 nm using a C-18 column. An aliquot (20 μ L) from the resulting derivatized samples was injected. The tyramine was detected in a gradient phase of 0.1 M ammonium acetate and ACN in a 90:10 ratio to 25:75 ratio as mobile phase for 10 min.

2.12 | Statistical analysis

The detection limit was calculated as the mean value of the LSPR peak intensity at zero concentration with three standard deviations (Oh et al., 2019). The statistical analysis was performed using OriginPro 9. All the results are presented as the average $\pm$ mean standard deviation of triplicates.

3 | RESULTS AND DISCUSSION

3.1 | Screening, identification, and characterization of tyramine-specific peptides by phage display

The phage display technique is an effective tool for screening and identifying peptides that bind to a target molecule. This is combinatorial technology in which random peptides are displayed on the surface of the phages. It has been widely exploited to recognize high-affinity peptides that bind to a target protein. The technique involves genetic insertion of gene of interest into the gene sequence of the viral surface protein. This enables the virus to display the recombinant gene as a surface protein which allows the screening of target-binding desired oligopeptides (Yun et al., 2019). The identified peptides can be used for substrate-specific targeting of several biomedical and biosensing applications (Bazan et al., 2012; Hwang et al., 2017; Li et al., 2017; Nemudraya et al., 2016; Park et al., 2010; Wu et al., 2016). The screening of tyramine-specific peptide sequence was done by the phage display technique as discussed in Section 2. The phage display technique was employed to screen peptides that selectively bound to tyramine (Figure 1a). Tyramine was conjugated with BSA (tyramine@BSA) to facilitate biopanning. Tyramine@BSA conjugates were analyzed using MALDI-TOF, confirming the successful conjugation of approximately 24 tyramine molecules to a single BSA molecule (Figure S1). Leveraging this complex, 12-mer affinity peptides were identified that selectively bind to tyramine@BSA conjugate from phage libraries. The nonspecific phage binding sites were eliminated through

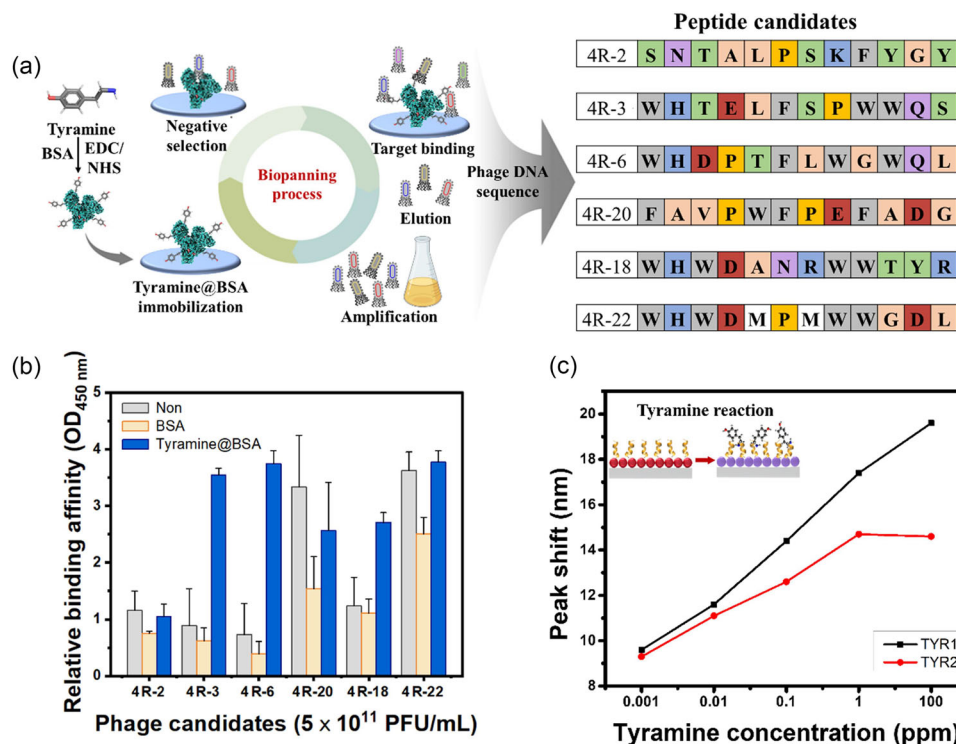


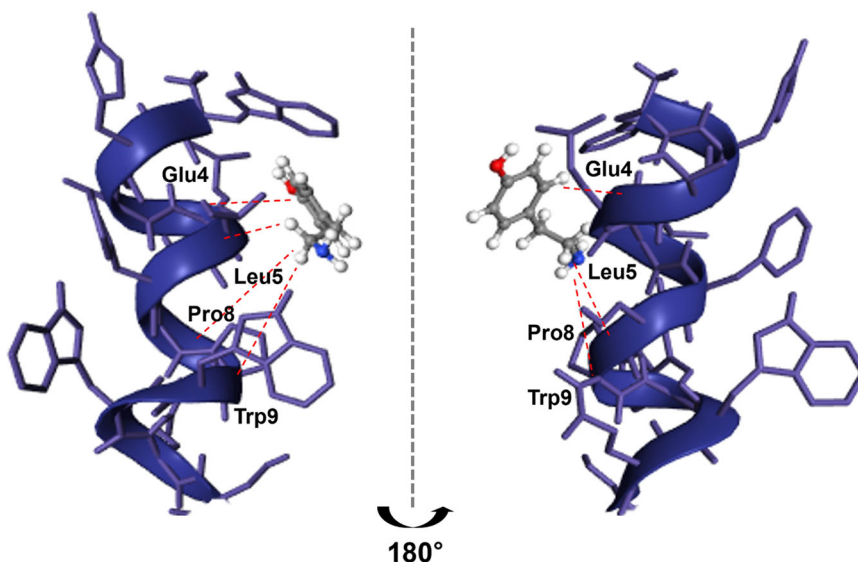
FIGURE 1 Screening of tyramine-specific binding peptides by phage display technique: (a) schematic representation of biopanning process and identification of six types of tyramine-specific peptides, (b) screening of suitable phage candidates for expressing the tyramine-specific binding peptides using enzyme-linked immunosorbent assay (ELISA) method, and (c) optimized localized surface plasmon resonance (LSPR) sensor chip detection of tyramine by using the TYR1 (4R-3) and TYR2 (4R-6) peptides.

negative selection for BSA to enhance the phage titers associated with tyramine binding (Figure S2). The biopanning process resulted in the sequencing of 12-mer peptides from 61 phages. Among them, only six unique peptide sequences were isolated as potential candidates for the targeted detection of tyramine (Figure S3).

The results from our phage display analysis showed that only six groups of phage candidates (4R-2, 4R-3, 4R-6, 4R-20, 4R-18, and 4R-22) were found for their specific binding affinity toward tyramine. Among these six phage groups, only two phage groups (4R-3 and 4R-6) have shown specifically higher relative binding affinity toward tyramine (Figure 1b). The tyramine-specific peptide sequences expressed in phage candidate 4R-3 and 4R-6 were sequenced and found to be WHTELFSPWWQS (TYR1) and WHDPTFLWGWQL (TYR2), respectively. The selection of specific peptides is crucial to ensure accurate recognition of target molecules in the peptide-applied detection sensor. As revealed by Figure 1b, the 4R-3 (WHTELFSPWWQS) and 4R-6 (WHDPTFLWGWQL) phages showed significantly higher binding affinity to tyramine@BSA conjugates, and with relatively low nonspecific binding compared to other phages groups. Interestingly, both selected peptides share a similar amino acid sequence motif, WHXE(D)LFSP(PTFL)WXWQX, as demonstrated in Figure S3b. This shared motif may play a critical role in conferring the observed high binding affinity for tyramine–BSA conjugates and could potentially be the key determinant in their specificity. The successful identification of these specific peptides, 4R-3, and 4R-6, with their distinct bind-

ing characteristics, holds significant promise for their application in detecting and recognizing target molecules accurately. The degree to which a peak shift occurred in between these peptides (TYR1 and TYR2) that reacted more specifically to tyramine was confirmed by the results from UV–visible spectrophotometric analysis. The peak shift phenomena observed in our study was corroborated with the peak shift phenomena reported by Li et al. (2009). The peak shift was observed due to the LSPR of AuNPs present on the chip. The LSPR of AuNPs has been reported to be affected by both the dielectric characteristics of their surroundings and the plasmon coupling. When the refractive index near the AuNPs increases, the LSPR spectrum experiences a shift toward longer wavelengths, commonly referred to as a red shift. Therefore, changes in the local refractive index due to specific molecular interactions or adsorption on the surface of AuNPs may lead to observable shifts in the LSPR peak (Li et al., 2009). The peak shift assay was conducted for TYR1 and TYR2 for the better binding affinity at various concentration of tyramine (1 ppb to 100 ppm). TYR1 was measured to have peak shifts of 17.9, 20.6, and 21.8 nm at 100 ppb, 1 ppm, and 100 ppm, respectively. In contrast, TYR2 was measured at 11.8, 14.4, and 14.6 nm over the same concentration range. The TYR1 peptide had shown higher relative binding affinity than the TYR2 peptide against tyramine (Figure 1c). The specific binding affinity of peptides was observed to be concentration dependent against standard tyramine. This indicated that TYR1 is more sensitive to tyramine detection in the LSPR sensor than TYR2. Therefore, TYR1 was selected as a

FIGURE 2 Molecular docking simulation of tyramine and TYR1 peptide using DockThor web server. Three-dimensional structures of TYR1–tyramine complex showing the binding sites.



more suitable peptide for tyramine detection and to be used in the fabrication of LSPR sensor.

3.2 | Molecular docking simulation for analysis for affinity binding of peptide and tyramine

The molecular docking simulations were conducted to evaluate the binding efficiency and interactions of TYR1 peptide with tyramine using the DockThor program, as described in Section 2. The findings of docking study illustrated the binding interfaces of Glu 4, Leu 5, Pro 8, and Trp 9 amino acid residues in TYR1 that interact with tyramine (Figure 2). Notably, Leu at position 5 displayed the smallest contact distance. The RMSD between Leu residues of TYR1 and tyramine was measured to be 1.180 Å, suggesting a potential strong binding affinity between this residue and tyramine. The Glu, Leu, Pro, and Trp residues in TYR1 were identified as key binding sites, consistent with established knowledge regarding peptide–ligand interactions. Hydrophobic and aromatic residues, such as Leu and Trp, are known to play crucial roles in facilitating ligand recognition and binding. The presence of Glu and Pro residues may further contribute to the stability and specificity of the TYR1–tyramine complex. The result from molecular docking study had supported our above experimental findings that the TYR1 peptide could be a potential candidate to be used for the fabrication of LSPR sensor for tyramine detection. However, the molecular docking serves as a simulation technique and lacks experimental validation. Therefore, these findings were experimentally validated and discussed in the following sections.

3.3 | Preparation and characterization of AuNPs

The growing nanotechnology offers an outstanding platform to develop approaches for real-time on-site detection of food spoilage and adulteration. Nanoparticles have been reported to exhibit better

electrical, optical, or catalytic properties than the materials. Among several nanoparticles, AuNPs have been reported as an excellent promising candidate for the development of biosensors. A slight change in the amount of salt in the medium can cause immense aggregation of AuNPs, which finally results into the change of color (Mirkin et al., 1996). The LSPR of AuNPs is affected by both the dielectric characteristics of their surroundings and the plasmon coupling between particles. When the refractive index near the AuNPs increases, the LSPR spectrum get shifted toward longer wavelengths. The phenomenon is known as a red shift. The changes in local refractive index due to specific molecular interactions on the surface of AuNPs can lead to observable shifts in the LSPR, which may be detected (Li et al., 2009). AuNPs (50 nm) were synthesized by following a previously reported Turkevich/Frens reaction system as discussed in Section 2. The HAuCl₄ was reduced by sodium citrate to generate spherical, monodispersed, and surface functionalized AuNPs in aqueous solutions (Kettemann et al., 2016; Larm et al., 2018). This nanomanufacturing method is highly reproducible, convenient, and cost-effective with least environmental impact (Larm et al., 2018). The synthesis of nanoparticles with desired size and shape has been reported to be very important (Sau et al., 2001). The shape and size of AuNPs may be modulated by nature of solvent system, particle stabilizer, and reaction conditions such as temperature and pH (Toubanaki et al., 2020). It has been reported that the size of AuNPs potentially affect the detection of analytes, as smaller nanoparticles provided a better detection range (Oh et al., 2017). Since AuNP size affects NP optical properties, it is important to synthesize an appropriate size of AuNPs to improve biosensor performance. Previous reports indicated that smaller AuNPs in LSPR sensor chips have resulted in more sensitive LSPRs by producing more hotspots for change the surface charge of the particle (Kim et al., 2018). If the particle size is too small there may be difficulty in visually identifying the detection area signal on the Lateral flow immunoassay (LFIA) strip. However, the AuNPs larger than 50 nm may cause unstable antigen–antibody binding due to steric hindrance. It has been reported previously that less than 50 nm AuNPs were highly

effective (Khlebtsov et al., 2019; Toubanaki et al., 2020). Therefore, it was imperative to synthesize smaller sized AuNPs for our LSPR biosensor development. Therefore, the synthesized AuNPs were analyzed by DLS-zeta sizer, SEM, TEM, and UV-visible spectrophotometer.

The results from DLS-zeta sizer analysis of our study showed that the average hydrodynamic size of AuNPs was found to be 28.8 nm. However, on the surface analysis of the synthesized AuNPs, the zeta potential was -11.5 mV (Figure S4a). The hydrodynamic size and zeta potential analysis of AuNPs have indicated that the results corroborated with the findings of several previous reports (Heo et al., 2017; Kim et al., 2017, 2018; Lee et al., 2022; Oh et al., 2017, 2019).

The AuNPs were preferred over other metals because of their distinct optical property and characteristics of having a strong absorbance between 500 nm and 600 nm. The uniform shape and size of the AuNPs has been reported to be very important for the reliability and reproducibility of the LSPR biosensors. The absorbance peak signals have been reported to characterize the shape and size of the AuNPs (Haiss et al., 2007; He et al., 2005). UV-visible absorption spectra analysis of AuNPs were measured in the wavelength ranges from 700 nm to 400 nm. The peak absorbance of the AuNPs was observed at a wavelength of 525.39 nm (Figure S4b), which is similar with the results of previous studies (Haiss et al., 2007; He et al., 2005). In another study, the synthesized AuNPs has been reported to exhibit peak absorption spectrum ($\lambda_{\max}$) at 520 nm (Oh et al., 2019). The results from TEM scanning of synthesized AuNPs have been found to be in support the findings from DLS and UV-visible spectrophotometric analysis. TEM image analysis has revealed that AuNPs have a uniform spherical shape and diameters of less than 50 nm (Figure S4c). Our findings have been corroborated with the findings of previously reported to synthesize the different sizes of AuNPs (Kim et al., 2017, 2018).

3.4 | Fabrication and characterization of antityramine cuvette-form LSPR sensor using TYR1 peptide

In this study, we developed a portable LSPR sensor chip with strong potential as a field detection device for the detection of tyramine in food samples. The glass chips on which AuNPs have been deposited were found to efficiently produce resonance phenomena. This resonance phenomenon depends on the incident light and the reaction conditions, such as the material type and thickness of glass chip, thus generating changes in the absorption spectrum that may function as a detection signal.

To fabricate the desired tyramine-specific LSPR sensor, the 8 mm $\times$ 50 mm size glass chips were used. The procedures for the fabrication of LSPR were mentioned in Section 2. The synthesized AuNPs (>50 nm) have been found to show an absorption maxima at 525.39 nm (Figure S4b) and have been in agreement with previous reports (Oh et al., 2019). However, when other substances are attached to the AuNPs by the LSPR effect, the refractive index of the surface changes, resulting in a peak shift that shifts the wavelength at which the maximum absorbance occurs to the right. The schematic representation of change in peak shift has been presented in Figure 3a.

Based on this principle, we fabricated a cuvette-form LSPR sensor for the detection of tyramine with high sensitivity using the tyramine-specific peptide (TYR1). The SEM analysis shown in Figure 3b indicated the confirmed uniform coating of AuNP on LSPR chip. The conjugation of TYR1 to AuNPs-coated LSPR chips was confirmed by peak shift study. As shown in Figure 3c, the bare chip coated with AuNPs has absorbance at 528 nm whereas the TYR1-conjugated LSPR chips were showing absorbance around 533.2 nm. This rise in absorption maxima of AuNP-coated LSPR chips indicated the presence of TYR1 peptide on it. The peak shift assay was conducted in the presence and absence of TYR1 peptide attached with AuNP-coated LSPR chips. The change in peak shift between bare chip and the AuNP-coated LSPR chips fabricated with different concentrations of TYR1 peptide had confirmed the binding of TYR1 peptide using different concentrations as the peak shift was found increasing with the TYR1 concentration (Figure 3d). The change in color was observed due to the aggregation of AuNPs present on the chip (Figure S5). The results of the peak shift assay were found to be corroborated with the previous findings (Kim et al., 2018; Oh et al., 2017). Our newly fabricated tyramine-specific LSPR sensor was found to be highly stable even after 30 days of storage (Figure S6).

3.5 | Optimization of TYR1 peptide immobilization on the LSPR chip and selective detection of tyramine

To optimize the tyramine-specific LSPR sensors were fabricated as discussed in Section 2. The LSPR sensor for tyramine detection was fabricated using less than 50 nm AuNPs. These AuNPs have a maximum absorbance of 535 nm. However, when peptides and target substances are attached to the AuNPs, the resonance around the AuNPs changes and a peak shift occurs, which can be used to selectively detect tyramine. The results from optimized LSPR sensors have been presented in Figure 4. TYR1 was allowed to react with the AuNPs-coated LSPR chips at the concentration range of 10–40 $\mu\text{g/mL}$ at different time intervals of 10 min (from 10 to 70 min). An optimum concentration of TYR1 showed maximum binding to the AuNPs-coated LSPR chip. The results showed the highest peak shift at 20 $\mu\text{g/mL}$ with a peak shift of 5.2 nm, which means that the most peptide was attached to the LSPR sensor at the peptide concentration of 20 $\mu\text{g/mL}$ (Figure 4a). The peptide reaction time was also optimized as it has a significant impact on peptide adhesion. The experimental results showed that the peak shift gradually increased up to 40 min, however, after 40 min, the peak shift no longer increased because the peptide was saturated (Figure 4b). The tyramine detection time was optimized using the UV-visible spectrophotometer at the different time intervals (5, 10, 20, and 30 min). The results from the present study indicated that our LSPR sensor has a 20 min detection time. This indicated that this sensor has a very quick response time against the tyramine detection (Figure 4c). The results from the specificity assay indicated that this sensor has greater affinity toward tyramine detection in comparison to cadaverine, histamine, methanol, ammonia, and ethanol. The sensor was found to be highly selective for tyramine, with a peak shift of over 86% compared to the other substances (Figure 4d).

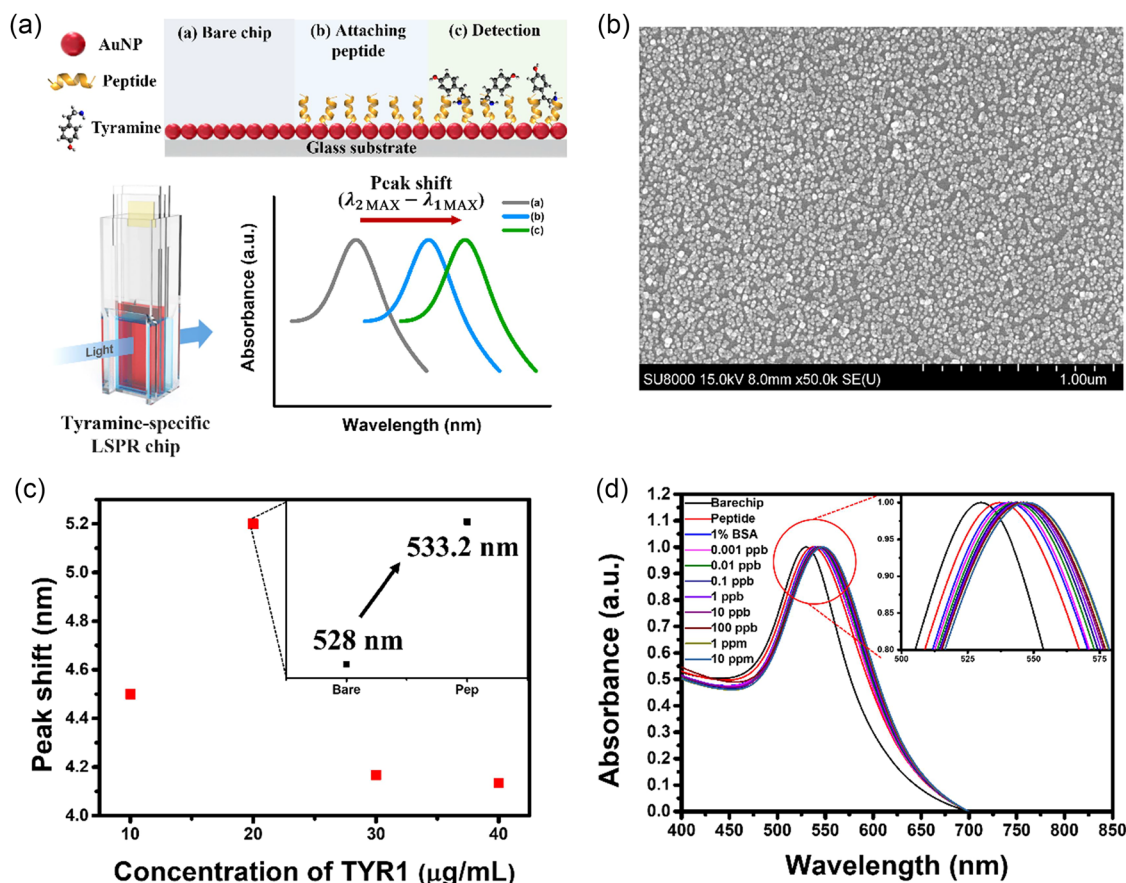


FIGURE 3 Fabrication and characterization of the TYR1-immobilized gold nanoparticles (AuNPs)-coated tyramine detecting localized surface plasmon resonance (LSPR) sensor. (a) Schematic representation, (b) SEM image of AuNP-coated LSPR chip, (c) the confirmation of TYR1 peptide conjugation on the surface of AuNP-coated LSPR chip by peak shift analysis, and (d) absorbance change and peak shift upon concentration-dependent addition of tyramine.

3.6 | Quantitative detection of tyramine by LSPR sensor in spiked samples of beef, mackerel, and cheese

In order to detect the tyramine concentration in food samples, they were spiked and processed. The procedures of sample preparation and tyramine detection through HPLC and LSPR device were the same as discussed in Section 2. The LSPR sensor detection was performed at different concentrations of tyramine range (0.001 ppb–10 ppm). The LOD and R^2 value was 0.001 ppb with 0.99942, respectively. The tyramine was detected in the spiked food samples of beef, mackerel, and cheese by freshly fabricated LSPR sensor. The results from the current study showed a linear graph in the concentration range of 0.01–10 ppb in the spiked samples of beef, mackerel, and cheese. However, LOD values for the spiked samples of beef, mackerel, and cheese were found to be 0.63 ppb, 0.46 ppb, and 0.27 ppb, respectively (Figure 5). All three samples were able to detect tyramine at concentrations lower than the detection limits published in other studies.

The HPLC detection was performed at different concentrations of tyramine (0.01, 0.1, 1, 2.5, 5, and 10 ppm). The results from HPLC (C-18 column) detection of tyramine showed 4.38 min of retention time.

The LOD and R^2 value was 262 ppb with 0.99943, respectively. However, the HPLC detection of our spiked food samples (beef, mackerel, and cheese) showed the R^2 values for tyramine were 0.99904, 0.99967, and 0.99946, respectively. The LODs for tyramine in beef, mackerel, and cheese were 105 ppb, 262 ppb, and 262 ppb under same conditions. The nonsignificant change in recovery rate of tyramine in case of beef, mackerel, and cheese at their different concentrations (10 ppm, 5 ppm, 2.5 ppm, and 0.1 ppm) was observed in present study. However, it showed significant change in the recovery rate of tyramine at lower concentration (0.1 ppm) in meat and fish sample (Table S1).

When comparing the results of HPLC and LSPR device, HPLC showed a lower sensitivity than LSPR sensor fabricated in this work (HPLC data given in Figure S7). This confirms that HPLC has a much higher LOD value than our LSPR sensor. The detection of tyramine using HPLC not only requires a long pretreatment and analysis time, but also has a much higher detection limit than the newly developed LSPR sensor, which confirms the limitations of HPLC in detecting tyramine at lower concentrations. The onsite detection of tyramine in the food samples may be possible by spiking the samples of beef, mackerel, and cheese using our LSPR sensor. These food products have been reported to contain and produce high levels of tyramine when

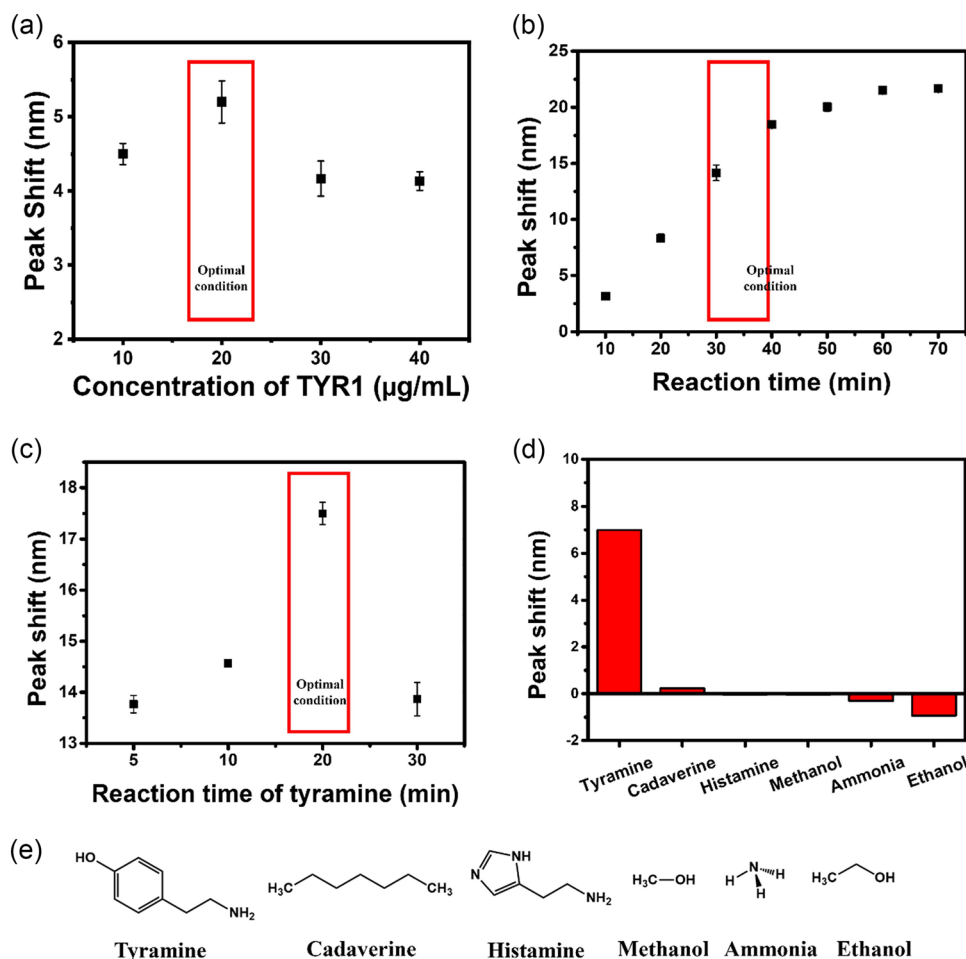


FIGURE 4 Optimization of fabricated localized surface plasmon resonance (LSPR) sensor: (a) Effect of different TYR1 peptide concentration (10, 20, 30, and 40 µg/mL) on the detection of tyramine, (b) Optimal reaction time of 20 µg/mL of TYR1 peptide concentration at different reaction times (10, 20, 30, 40, 50, 60, and 70 min), (c) Detection of tyramine at different reaction times (5, 10, 20, and 30 min), (d) Evaluation of selective sensitivity of optimized LSPR sensor toward tyramine, cadaverine, histamine, methanol, ammonia, and ethanol, and (e) The structure of tyramine, cadaverine, histamine, methanol, ammonia, and ethanol.

get spoiled. The results from current study indicated that the HPLC detection of tyramine is very time consuming and costly and needs long and difficult sample preparation procedures. In comparison to the complicated and time-consuming sample preparation process in HPLC detection method, our sensor showed very simple sample preparation steps and quick detection. The sample preparation for LSPR detection was achieved by placing three sizes of silica beads in a bottle along with sample and mixed with DMSO followed by filtration. This process took less than 5 min, dramatically reducing the sample preparation time compared to the sample preparation for HPLC.

The development of point-of-care sensors for identifying food spoilage is a crucial process. In a study, an amperometric biosensor was developed for the measurement of tyramine in food samples. The optimized detection reported to exhibit a linear response in the range of 10–120 µM with the LOD value of 0.71 µM for tyramine (da Silva et al., 2019). In another investigation, the colorimetric immunoassay was developed for tyramine detection in beef, pork, and yoghurt. It has been reported that the development of color is due to the shift in the

LSPR band of AuNPs. The LOD for tyramine detection in beef, pork, and yoghurt was 19.7 mg/kg which had been validated by HPLC (Luo et al., 2022). However, in present investigation, the actual samples of three different matrix were pretreated without any instrument for detecting tyramine and food spoilage factor at very low concentrations. The recovery rate of tyramine in the actual sample test was indirectly confirmed by HPLC (Figure S5). The spiked food samples were found to contain an amount of tyramine equal to the standard sample which showed that tyramine was not lost during the pretreatment process.

The SPR- and LSPR-based colorimetric techniques played an important role in on-site biosensing of food spoilage. Among them the LSPR has been reported to be the most efficient and very promising method for the in situ analysis because they are rapid, cost-effective, easily operated for onsite testing (Gao et al., 2017; Radhakumary & Sreenivasan, 2011; Unser et al., 2015). The SPR detection involved a collective oscillation of valence electrons present in the surface of solid metals excited by incident light. This process produces a nonradiation surface electromagnetic waves that propagate their energy in

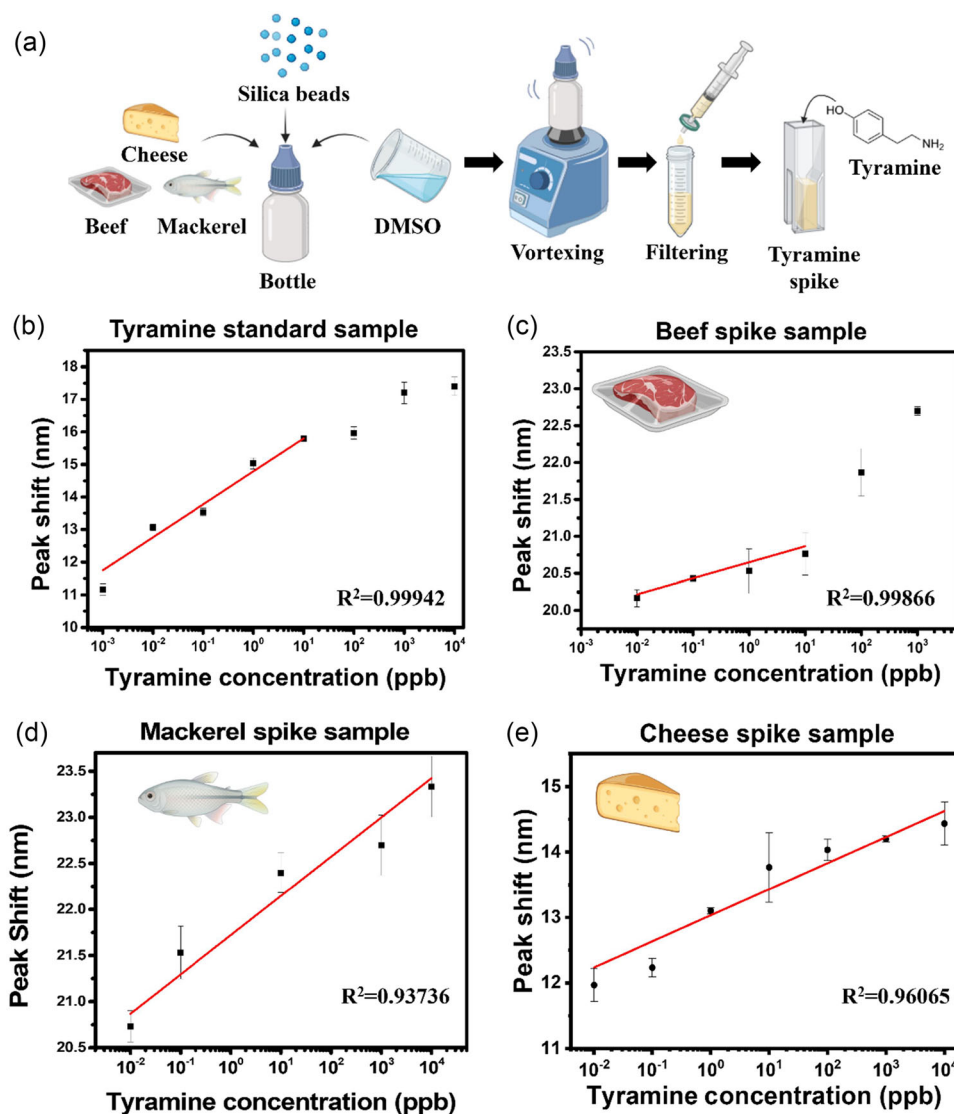


FIGURE 5 Detection of tyramine through optimized localized surface plasmon resonance (LSPR) sensor chip: (a) Schematic principle of experiment; (b) Detection of tyramine in standard solution of tyramine; and the spiked samples of (c) beef; (d) mackerel; and (e) cheese.

parallel to solid-dielectric interface but disperse in perpendicular direction (Abbas et al., 2011; Wang et al., 2021). In contrast, the LSPR has been related to produce resonance for detection at nanoscale and is a nonpropagating electromagnetic wave (Chu & Crozier, 2009). The SPR-based sensors respond to changes in refractive index when target analytes attach to the plasmonic surface. However, LSPR sensors respond to both change in refractive index and peak shift due to the aggregation of nanoparticles (Kim et al., 2018; Oh et al., 2017, 2019).

4 | CONCLUSION

In this study, we optimized the fabrication of plasmonically active strips using various transparent substrates and developed a cuvette-based LSPR sensor chip for possible field-ready applications such as point-of-care testing. A highly sensitive plasmonic-active biode-

vice was developed to detect tyramine (a BA) produced due to the spoilage of food by the activity of a variety of pathogenic bacteria responsible for food poisoning. AuNPs (average diameter of 50 nm) were deposited uniformly on a transparent glass substrate and the developed AuNPs-based plasmonic-active sensing chips were then conjugated successfully with tyramine-specific peptides by a simple dipping adsorption method. The functionalized LSPR sensing chips exhibited high-sensitivity, excellent selectivity, and great stability, even in the presence of different background microorganisms. The developed chip also produced a rapid (within 30 min) detection of tyramine in spiked food samples such as beef, mackerel, and cheese with the great range of detection limit. Thus, we confirmed the practical applicability of the developed LSPR sensing chips in identifying food spoilage in real food samples. The developed method may be used for a wide range of food samples spoilage with tyramine production as a byproduct of degradation in food sample. However, the applicability of the

developed method needs to be established in different food matrices before its commercialization. Overall, this developed LSPR sensing chip has potential applications to detect the byproduct (tyramine) of food degradation in the food industry, and for environmental monitoring. The LSPR chip detection platform developed in this study thus offers a new direction for portable sensors that can be used in the field of environmental monitoring, and food safety.

AUTHOR CONTRIBUTIONS

Soobin Han: Conceptualization; methodology; software; investigation; writing—original draft; writing—reviewing and editing. **Ji Hong Kim:** Writing—original draft; methodology; software; investigation. **Vivek Kumar Gupta:** Writing; methodology; software; investigation. **Ju Eun Bae:** Writing—original draft; methodology; software; investigation. **Chae Hwan Cho:** Methodology; software; investigation. **Hanseung Kim:** Methodology; software; investigation. **Yonghyeon Park:** Methodology; software; investigation. **Mi-Hwa Oh:** Resources; methodology; writing—original draft; writing—reviewing and editing. **Jong Pil Park:** Methodology; software; investigation; writing—original draft; writing—reviewing and editing. **Yun Suk Huh:** Supervision; conceptualization; methodology; software; investigation; writing—original draft; writing—reviewing and editing.

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CONFLICT OF INTEREST STATEMENT

The authors confirm that they have no conflict of interest to declare for this publication.

ETHICS STATEMENT

This article does not include any human or animal experiments.

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