



Prism-Coupled SPR Sensors for Rapid Halal and Label-Free Identification of Gelatin from Multiple Animal Sources

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Abstract

Rapid and reliable identification of gelatin origin is critically important for food authentication and halal verification. This study proposes a label-free optical sensing approach based on surface plasmon resonance (SPR) for discriminating bovine, porcine, and fish gelatin. Gelatin solutions (1–8% w/v) were analyzed using a prism-coupled attenuated total reflection (ATR)-SPR configuration, where resonance angle shifts were extracted from reflectance minima. Complementary finite-difference time-domain (FDTD) simulations were performed to validate experimental trends and elucidate field interactions at the metal–dielectric interface. Results show distinct refractive index (RI) profiles and concentration-dependent resonance shifts for each gelatin type, enabling clear differentiation without chemical labeling. At higher concentrations (7–8%), fish gelatin exhibits the highest RI, followed by porcine and bovine gelatin. Porcine gelatin demonstrates the most linear and sensitive response, with a strong correlation ($R^2 = 0.9412$) between concentration and resonance angle shift. Simulation results are in good agreement with experimental data, confirming the sensitivity of SPR to subtle RI variations. The novelty of this work lies in the integration of ATR-SPR and numerical modeling for rapid, non-destructive, and label-free identification of gelatin sources across multiple animal origins. This approach offers a promising platform for real-time food authentication, quality control, and halal assurance, with potential for portable and on-site sensing applications.

Keywords: FDTD simulation, gelatin, halal verification, surface plasmon resonance (SPR), refractive index (RI)

1. INTRODUCTION

The detection methods without labels, such as surface plasmon resonance (SPR), are particularly significant in the context of the biochemical analysis related to food security [1]. With the increase in global concern for adulteration and authenticity of food [2], the ability to analyze food products without the need for labeling agents improves the efficiency and reliability of detection methods [3]-[5]. Specifically, in the context of animal-derived products, there is a growing emphasis on verifying the composition of gelatins from cattle, pig, and fish [6][7]. The use of different types of gelatins has generalized implications in various industries, including food processing, pharmaceutical products and cosmetics, where

gelatin serves as a jellifying, thickening and stabilizing agent. The ability to detect and differentiate with precision between these gelatins is essential not only to guarantee product quality and compliance with regulatory standards, but also to address ethical and dietary concerns associated with animal supply.

Currently, the use of gelatin in the food and pharmaceutical industries is very massive [8]. Some countries are concerned about the source of gelatin due to the halal concept [6]. Therefore, gelatin measurement with an SPR sensor can complement and serve as an alternative to existing gelatin identification measurement systems. Conventional halal authentication methods, such as ELISA and PCR, often require longer processing times, expensive reagents, and destructive sample preparation [9]. In contrast, the prism-coupled SPR platform provided rapid (<10 min) and real-time detection without the need for chemical labeling. A reported detection limit (LOD) of 0.07 mg/mL has been shown to be comparable or superior to conventional immunoassay-based approaches. Moreover, the label-free nature of the detection reduces both cost and complexity, making the system more feasible for routine halal authentication [10][11].

SPR-based sensors operate based on the

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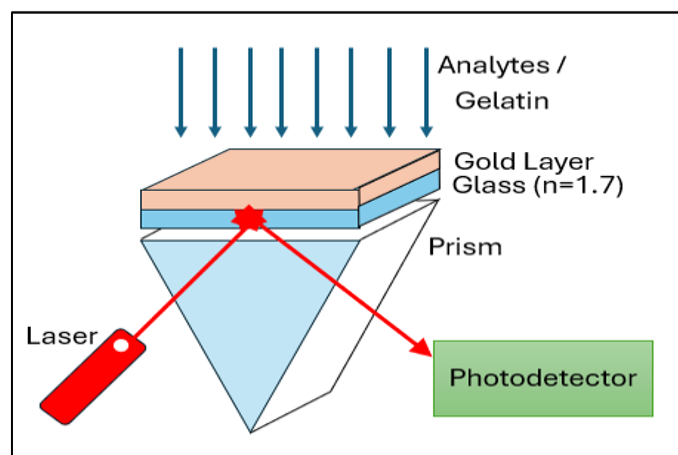


Figure 1. Measurement scheme of the prism-based SPR sensor with Kretschmann configuration for gelatin detection.

interaction of p-polarized light with free electrons at a metal–dielectric interface, enabling precise monitoring of analyte-induced optical changes [12]. Recently, various SPR-based configurations have been developed, including prism-coupled systems, photonic crystal fiber (PCF) sensors, and metal–insulator–metal (MIM) structures [13]. Meanwhile, the purposes of SPR-based measurements are also diverse, such as biological analytes, environmental related or bacterial detection in the health sector [14]. In halal perspective, the ability to differentiate porcine gelatin from bovine and fish gelatin is highly significant. The resonance shifts observed provide a unique optical “fingerprint” for each gelatin source. While further optimization, including molecular recognition elements (e.g., aptamers or antibodies), may enhance selectivity, the current results demonstrate that prism-based SPR sensors can already serve as a rapid screening tool for halal verification [15]. This has potential applications in food quality control, pharmaceutical authentication, and regulatory compliance where the presence of porcine derivatives must be strictly monitored [16].

Although the results are promising, some limitations must be addressed. The sensor performance may vary in complex food matrices where other proteins or additives can interfere with the resonance signal [17]. Further studies are needed to test real commercial samples and assess reproducibility under different environmental conditions. Integration with microfluidic channels and multiplexing capabilities could also enhance

throughput and applicability. The purpose of this research is to evaluate the effectiveness of optical waveguide [18] such as prism-based SPR sensor for detecting and differentiating bovine, porcine, and fish gelatin across various concentrations. Specifically, the study aims to (i) investigate the optical responses—such as refractive index variation, SPR resonance angle shifts, and sensitivity—for each gelatin type, (ii) compare experimental measurements with simulation results to validate sensor performance, and (iii) assess the potential of the SPR platform as a rapid, label-free, and reliable method for gelatin authentication, including applications in halal verification, food quality control, and pharmaceutical screening.

2. MATERIALS AND METHODS

The research employed both experimental and simulation methods. The experimental approach was used to examine the SPR response to gelatin solutions with varying concentrations. In the experimental setup, NanoSPR 6 was used based on the Kretschmann configuration, which consists of a glass prism, a gold film, and the gelatin solution. The sensor chip (a 5/45 nm Cr/Au layer on glass) was mounted onto the prism using immersion oil with a refractive index matching that of the glass. The gelatin solution flows across the gold thin film [19], as illustrated in Figure 1. A 650-nm p-polarized laser beam is directed toward the gold surface, exciting a collective oscillation of free electrons when the resonance angle is reached.

Although the incident light undergoes total internal reflection within the prism, its evanescent field penetrates the gold layer. This interaction is detected as a drop in reflectance (the minimum reflectance) at the SPR angle (θ_{SPR}). The reflectance was recorded using a photodiode through the ATR technique.

Analyte preparation was conducted by preparing gelatin solutions (bovine, porcine, and fish) at concentrations of 1% to 8%. The gelatin used was high-purity powdered gelatin from Sigma-Aldrich. Refractive index measurements were obtained using a refractometer. To validate the measurement results using a prism-based SPR sensor, we characterized the analytes in the form of gelatin (bovine-porcine-fish) using Fourier-transform infrared (FTIR) spectroscopy to distinguish between chemical compounds and functional groups of the molecules, and refractive index measurement [20].

In the FDTD simulation, the SPR sensor model consists of a dielectric/Au/analyte multilayer, in which the plasmonic layer is a 50-nm gold film. The dielectric prism is assumed to be silica with a

refractive index of 1.51, and the gold permittivity values are taken from Johnson and Christy [21]. A 650-nm light source with the same polarization mode as the experimental SPR setup is used. A DFT transmission monitor is employed to track the transmitted field profile, and perfectly matched layers (PML) are applied as boundary conditions [21]. To simulate the SPR response for glucose detection, the refractive index of gelatin at concentrations from 1% to 8% was used.

3. RESULTS AND DISCUSSIONS

3.1. FTIR Characterizations

The FTIR spectra in Figure 2(a) – 2(c) show a broad absorption band between 3000–3400 cm^{-1} , corresponding to the N–H stretching vibration (amide A) of the protein. The band in the 1600–1700 cm^{-1} region represents the amide I vibration associated with peptide [6]. In this study, the amide A peaks appear at 3298, 3306, and 3308 cm^{-1} for bovine, fish, and porcine gelatin, respectively. Meanwhile, the amide I peaks are observed at 1641, 1641, and 1639 cm^{-1} for bovine, fish, and porcine

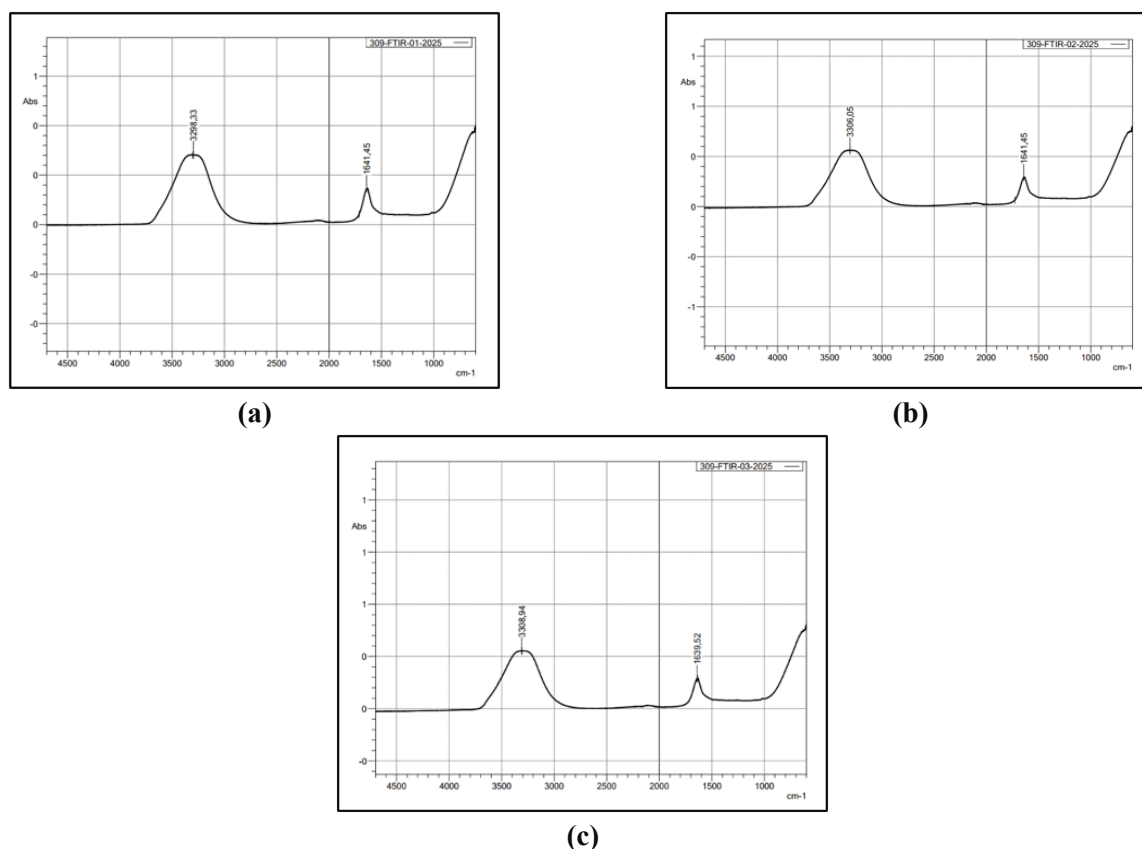


Figure 2. FTIR spectra of (a) fish, (b) bovine, and (c) porcine gelatin.

Table 1. Refractive indices of gelatin depend on the concentration.

Concentration of Gelatin	Refractive Index		
	Bovine	Porcine	Fish
1%	1.33431	1.33418	1.33432
2%	1.33563	1.33585	1.33556
3%	1.33655	1.33711	1.33527
4%	1.33832	1.33844	1.3385
5%	1.33996	1.33861	1.34001
6%	1.34114	1.34169	1.34175
7%	1.34291	1.34136	1.34346
8%	1.34440	1.3446	1.34467

gelatin. These differences indicate variations in the molecular structure and functional group organization among bovine, fish, and porcine gelatin.

3.2. Refractive Index Measurement

Refractive index (RI) measurements are performed to identify substantive differences between the analytes used in the measurements. Measuring the RI with a refractometer is one way to determine the optical properties of the analytes used in the measurements, as shown in Table 1. The RI values of bovine, porcine, and fish gelatin show distinct trends as gelatin concentration increases from 1% to 8%, reflecting differences in molecular composition, hydration behavior, and structural stability among the three gelatin types. Bovine gelatin exhibits a generally increasing refractive index with concentration, rising from 1.33431 at 1% to 1.34440 at 8%. This consistent upward trend suggests that bovine gelatin forms a relatively stable sol-gel network as concentration increases, resulting in predictable enhancements in mass density and optical polarizability. The smoothly increasing RI indicates that bovine gelatin maintains uniform hydration and molecular organization, consistent with its well-known structural stability derived from a higher content of proline and hydroxyproline. These properties support its typical use in applications requiring consistent and controllable optical characteristics.

Porcine gelatin also shows an overall increase in refractive index with concentration, but with slight fluctuations. The RI rises from 1.33418 at 1% to

1.34460 at 8%, with a small dip at 5–7% concentration. This deviation may result from concentration-dependent changes in molecular packing or hydration. Porcine gelatin is known for its relatively flexible chain structure, which can undergo subtle rearrangements in semi-dilute regimes. Despite these minor variations, porcine gelatin still demonstrates a strong final RI enhancement comparable to bovine gelatin, indicating that it remains highly responsive to concentration changes. This behavior aligns with the higher sensitivity observed in the SPR angle-shift results.

Fish gelatin shows the most dynamic and steep increase in refractive index among the three types. Starting from 1.33432 at 1%, the RI rises sharply to 1.34467 at 8%, surpassing both bovine and porcine values at higher concentrations. Fish gelatin's more pronounced RI enhancement can be attributed to its lower amino acid content, which typically results in higher flexibility and greater hydration capacity. As concentration increases, water is more readily displaced, leading to a faster increase in the effective optical density of the solution. The nonlinear behavior from 2% to 5%—where fish gelatin temporarily dips before rising sharply again—may reflect transient structural rearrangements or gelation processes that alter its polarizability. At low concentrations (1–3%), all gelatin types exhibit similar RI values, with minor differences within ± 0.0003 . At intermediate concentrations (4–6%), fish gelatin begins to surpass the others, especially beyond 5%. At high concentrations (7–8%), fish gelatin shows the

highest refractive index, followed by porcine and bovine gelatin.

3.3. ATR Curve of the SPR Measurement

Figure 3 shows the ATR-based SPR reflectance curves for 1% fish, bovine, and porcine gelatin. Each curve exhibits a characteristic reflectance minimum representing the resonance angle at which surface plasmon excitation occurs. The position and shape of these minima provide insights into the refractive index behavior and interfacial interactions of the different gelatin types. Fish gelatin demonstrates the leftmost resonance dip at $\sim 65^\circ$, which is both broader and shallower than those of the other samples. The broader linewidth indicates reduced plasmon confinement and increased optical damping at the metal–gel interface. This behavior aligns with the known structural properties of fish gelatin, which generally contains lower proline and hydroxyproline content, leading to less compact molecular packing and greater hydration. These characteristics reduce its effective refractive index, resulting in a lower SPR angle and diminished resonance sharpness.

In contrast, bovine gelatin exhibits a deeper and sharper resonance dip centered $\sim 67^\circ$. The narrower linewidth suggests more efficient plasmon coupling and reduced damping losses. This improved resonance is likely associated with the more stable triple-helix–derived structure and higher amino acid content of bovine gelatin. These properties may contribute to better surface coverage and a more uniform dielectric environment, leading to stronger

and more well-defined plasmon excitation. Porcine gelatin displays the highest resonance angle ($\sim 68.5^\circ$), indicating the largest effective refractive index at identical concentration. The reflectance minimum is smooth and symmetric, reflecting stable interaction with the metallic surface and consistent interfacial organization. The elevated resonance angle suggests that porcine gelatin induces the greatest refractive index contrast, even at low concentration, supporting its suitability for sensitive refractive-index–based detection. Collectively, the differences in resonance angle, dip depth, and linewidth highlight the unique optical signatures of each gelatin type and demonstrate the capability of ATR-based SPR to distinguish gelatin sources based on their interfacial optical properties.

Figure 4 shows the SPR angle-shift measurements provide insights into how gelatin from different biological sources interacts with the metal surface and how its refractive index changes with concentration. The SPR angle-shift measurements obtained for fish, bovine, and porcine gelatin show clear differences in their optical responses as a function of concentration. These variations reflect both the refractive index behavior of each gelatin type and their molecular interactions near the metal–dielectric interface. At low concentrations, fish gelatin exhibits the highest initial angle shift, suggesting a strong interaction with the plasmonic surface. However, its angle shift decreases at 2% before gradually stabilizing at higher concentrations. This non-monotonic trend indicates that fish gelatin undergoes structural or

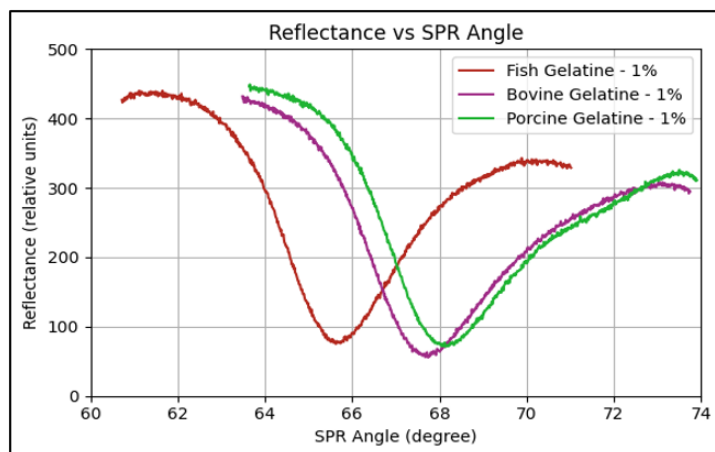


Figure 3. SPR reflectance curves of 1% fish, bovine, and porcine gelatin obtained using the ATR configuration.

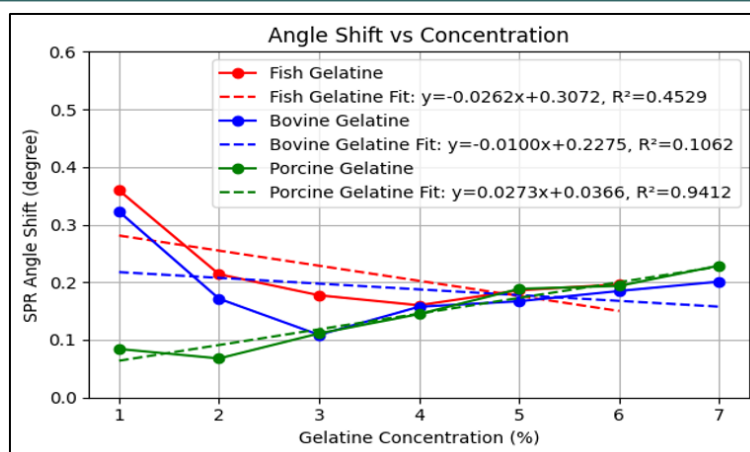


Figure 4. SPR angle shift for three types of gelatins at varied concentrations.

hydration-related changes that reduce its effective refractive index contribution as concentration increases. In contrast, bovine gelatin shows the most stable SPR response, with minimal variation in angle shift across the entire concentration range. This consistency is likely associated with its relatively uniform triple-helix-derived molecular structure and higher intrinsic stability compared with fish gelatin.

Porcine gelatin displays a markedly different pattern, characterized by a steady increase in angle shift from 1% to 7%. This behavior suggests that porcine gelatin exhibits a more pronounced concentration-dependent refractive index enhancement. Among the three samples, porcine gelatin demonstrates the strongest overall response to concentration variation. The sensitivity of the SPR system to gelatin concentration, represented by the slope of the linear regression, further highlights these differences. Fish gelatin shows a negative sensitivity ($-0.0262^\circ/\%$), consistent with its decreasing angle shift at higher concentrations. Bovine gelatin also exhibits a negative but smaller slope ($-0.0100^\circ/\%$), confirming its low concentration sensitivity. Porcine gelatin presents the highest positive sensitivity ($+0.0273^\circ/\%$), indicating the strongest SPR response per unit concentration. These results suggest that porcine gelatin provides the most distinct and measurable optical changes within the tested range.

Linearity analysis reinforces this conclusion. Porcine gelatin yields an R^2 value of 0.9412, indicating excellent linear correlation between concentration and angle shift. Fish gelatin shows moderate linearity ($R^2 = 0.4529$), reflecting its non-

monotonic pattern. Bovine gelatin presents lowest linearity ($R^2 = 0.1062$), further supporting the observation that its angle shift remains relatively constant across concentrations. Overall, these findings demonstrate that each gelatin type exhibits a unique SPR signature. Porcine gelatin is the most responsive and predictable, bovine gelatin is the most stable but least sensitive, and fish gelatin shows the most complex behavior. These differences highlight the potential of SPR as a rapid and label-free method for distinguishing gelatin sources based on their concentration-dependent optical properties.

3.4. Simulation Results

Figures 5 and 6 present the simulated SPR responses of bovine, porcine, and fish gelatin as functions of gelatin concentration and refractive index, respectively. The simulation outputs show highly linear relationships, with R^2 values consistently above 0.92 for all gelatin types—substantially higher than those observed in the experimental data. This high linearity indicates that the simulation model accurately captures the optical behavior of gelatin films when idealized conditions (uniform thickness, homogeneous refractive index distribution, and noise-free environment) are assumed. In Figure 5, the SPR angle increases proportionally with gelatin concentration for all samples. Fish and porcine gelatin share identical slopes ($0.226^\circ/\%$), suggesting similar effective refractive index increments per concentration unit, while bovine gelatin exhibits a slightly higher slope ($0.242^\circ/\%$). This implies that, at equal concentrations, bovine gelatin introduces a

marginally stronger perturbation in the evanescent field, likely due to differences in molecular density or hydration behavior. The close clustering of data points and high R^2 values (0.9268–0.9524) confirm that concentration is a robust predictor of SPR angle shift in the simulation domain.

Figure 6 further validates this trend by correlating SPR angle with refractive index. All gelatin types display clear linear dependencies, with porcine gelatin showing the strongest linearity ($R^2 = 0.9967$), followed by bovine ($R^2 = 0.9383$) and fish ($R^2 = 0.9512$). The slopes indicate sensitivity to refractive index changes, with bovine gelatin yielding the highest simulated sensitivity ($0.0018^\circ/\text{RIU}$), compared to porcine ($0.0016^\circ/\text{RIU}$) and fish ($0.0014^\circ/\text{RIU}$). These differences reflect subtle variations in optical contrast and film-metal interface interactions among the gelatins. The strong agreement between increasing refractive index and resonance angle shift reinforces the accuracy of the SPR principle in distinguishing gelatin types based on their optical properties.

The simulation results show consistent and predictable SPR responses, highlighting the sensitivity of the technique to both concentration and refractive index variations. The high degree of linearity demonstrates that, under controlled conditions, SPR can reliably differentiate gelatin sources. Although the simulation results suggest that bovine gelatin exhibits the highest intrinsic SPR sensitivity, the experimental results show that porcine gelatin provides the strongest and most consistent response. This discrepancy arises from the difference between idealized simulation

conditions and real experimental environments. Simulations assume homogeneous RI, uniform film thickness, and noise-free conditions, reflecting intrinsic optical properties. In contrast, experiments are affected by surface non-uniformity, hydration effects, interfacial interactions, and instrumental noise, which influence plasmonic coupling and the effective RI. Therefore, simulations serve as a theoretical benchmark, while experimental results represent practical sensor performance. The consistent overall trends confirm that both approaches are complementary.

The experimental and simulation results exhibit consistent trends, with both showing that increases in gelatin concentration and RI lead to corresponding increases in the SPR resonance angle for all gelatin types. Experimental measurements display greater variability and smaller angle shifts, influenced by factors such as film non-uniformity, hydration effects, and instrumental noise, whereas the simulations yield highly linear responses due to idealized and uniform model assumptions. Despite these differences in magnitude and smoothness of response, both datasets indicate that bovine gelatin provides the highest SPR sensitivity, followed by porcine and fish gelatin. The close agreement in trend between experiment and simulation supports the reliability of the SPR mechanism for differentiating gelatin types.

It is noted that the study was conducted using pure gelatin solutions under controlled conditions, which may not fully represent complex real-world samples. In practical applications, food and pharmaceutical matrices contain components such

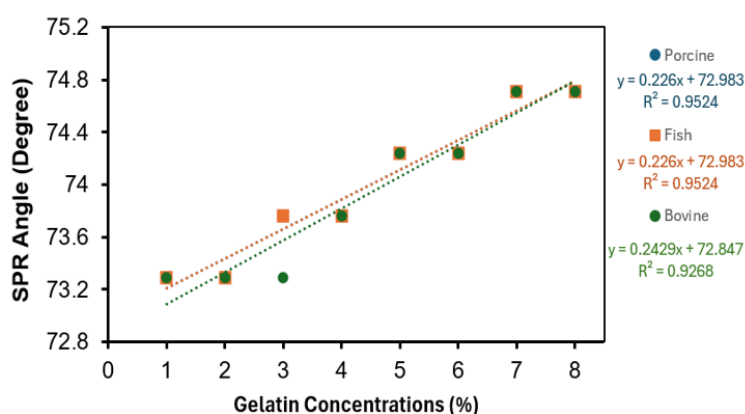


Figure 5. Simulated SPR angle as a function of gelatin concentration (1–8%) for bovine, porcine, and fish gelatin.

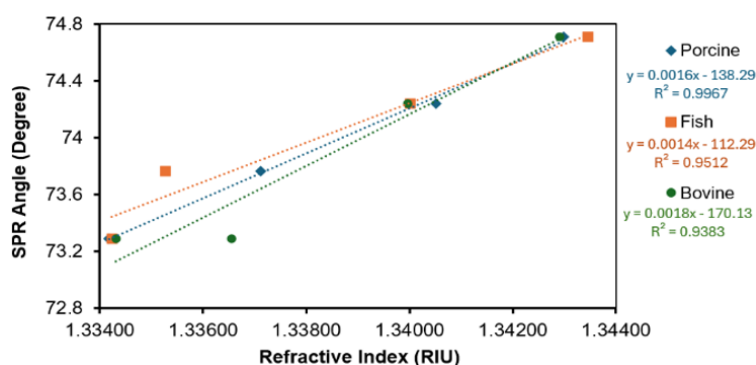


Figure 6. Simulated SPR angle as a function of refractive index for bovine, porcine, and fish gelatin.

as sugars, lipids, proteins, and additives that can alter the refractive index and introduce non-specific interactions, potentially affecting the SPR response. Nevertheless, the use of pure gelatin provides a necessary baseline to understand the intrinsic optical behavior of different sources. Future work will focus on validating the sensor using real samples, integrating microfluidic systems, incorporating selective recognition elements and cross-validating with established methods.

4. CONCLUSIONS

The SPR characterization of bovine, porcine, and fish gelatin demonstrates that all three materials exhibit a clear and concentration-dependent increase in RI, which correlates with systematic resonance angle shifts in the ATR-SPR curves. Among the samples, bovine and fish gelatin show more consistent RI value with concentration, whereas porcine gelatin displays slight irregularities at mid-range levels, indicating structural or compositional differences that affect its optical response. ATR-based SPR measurement showed that porcine gelatin exhibits the most stable and predictable response. The R^2 value of 0.9412 demonstrates an excellent linear correlation between concentration and angle shift. The simulation results also reveal consistent and reliable SPR responses. These findings collectively indicate that SPR can effectively distinguish gelatin types based on their refractive index profiles and angle-shift behavior, supporting its potential use in gelatin identification, quality monitoring, and halal authentication applications.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article. The funding sources had no involvement in the study design, data collection, analysis, interpretation, or manuscript preparation.

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DECLARATION OF GENERATIVE AI

The authors used AI-assisted tools solely for language editing and formatting purposes. No generative AI tools were used for data analysis, interpretation of results, or generation of scientific conclusions. The authors retain full responsibility for the accuracy and integrity of the work.

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