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# *E. coli* O157:H7 Detection Using Surface Plasmon Resonance Based Biosensor

# Yüzey Plazmon Rezonans Temelli Biyosensör ile *E. coli* O157:H7 Tespiti

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## ABSTRACT

The detection of foodborne pathogenic bacteria remains a significant challenge, and the need for fast and sensitive detection methods is becoming increasingly important. *Escherichia coli* is a prevalent bacteria associated with foodborne illness, and this study aimed to evaluate the ability of a surface plasmon resonance (SPR) based biosensor to detect *E. coli* O157:H7 at low levels in pure culture and artificially contaminated bay leaves (*Laurus nobilis*) using different injection methods. To develop a biological sensing surface, the sensor surface was functionalized with 3-aminopropyltriethoxysilane (APTES), and polyclonal antibodies were immobilized on the surface for bacteria detection. Bacterial attachment to the antibodies resulted in a change in resonance angle. The biosensor was able to discriminate between cellular concentrations of $10^3$ to $10^7$ CFU/mL and showed potential in detecting different pathogens in various food samples. Before the SPR detection, the sample preparation step was optimized to ensure complex food matrices were suitable for SPR analysis. Additionally two different injection ports were compared to investigate the impact of flow rate on binding events at the sensor surface. In one of these ports, solutions were pumped onto the sensor surface at a flow rate of 1.6 µL/s, while in the other method, without any flow, a volume of 10 µL of sample was transferred to the chip surface using a pipette and kept for 10 minutes, after which the signal dynamics were examined. The results suggest that the SPR based biosensor is a promising tool for the rapid detection of foodborne pathogens in complex food matrices.

**Key Words**

*E. coli* O157:H7, SPR based biosensors, Bacterial detection, *Laurus nobilis*.

## ÖZ

Gıda kaynaklı patojenik bakterilerin tespiti önemli bir zorluk olmaya devam etmektedir ve hızlı ve hassas tespit yöntemlerine olan ihtiyaç giderek daha zorunlu hale gelmektedir. *Escherichia coli*, gıda kaynaklı hastalıklarla ilişkili yaygın bir bakteridir ve bu çalışma, yüzey plazmon rezonansı (SPR) temelli bir biyosensörün, saf kültürde ve yapay olarak kontamine olmuş defne yapraklarında (*Laurus nobilis*) düşük seviyelerde E. *coli* O157:H7'yi farklı enjeksiyon yöntemleri kullanarak saptama yeteneğini değerlendirmeyi amaçlamıştır. Biyolojik bir algılama yüzeyi geliştirmek için sensör yüzeyi, bakteri tespiti için 3-aminopropiltrietoksisilan (APTES) ile işlevselleştirilmiş ve yüzey üzerine poliklonal antikorlar immobilize edilmiştir. Antikorlara bakteriyel bağlanma, rezonans açısında bir değişikliğe neden olmuştur. Biyosensör, $10^3$ ile $10^7$ CFU/mL arasındaki hücresel konsantrasyonları ayırt ederek ve çeşitli gıda örneklerinde patojenleri tespit etme potansiyeli göstermiştir. Ayrıca SPR tespitinden önce, karmaşık gıda matrislerinin SPR analizi için uygun olmasını sağlamak için numune hazırlama adımı optimize edilmiştir. Ek olarak, akış hızının sensör yüzeyindeki bağlanma olaylarına etkisinin karşılaştırılması için iki farklı enjeksiyon portu kullanılmıştır. Bu portlardan birinde 1,6 µL/s akış hızında solüsyonların sensör yüzeyine pompalanmasına imkan tanınırken diğer yöntemde herhangi bir akış söz konusu olmadan 10 µL hacmindeki örnek pipet yardımı ile çip yüzeyine aktarılıp 10 dk bekletilerek ve sinyal dinamikleri incelenmiştir. Elde edilen sonuçlar, SPR tabanlı biyosensörün, karmaşık gıda matrislerinde gıda kaynaklı patojenlerin hızlı tespiti için umut verici bir araç olduğunu göstermektedir.

**Anahtar Kelimeler**

*E. coli* O157:H7, SPR tabanlı biyosensörler, Bakteri tespiti, *Laurus nobilis*.

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## INTRODUCTION

*E. coli* is known as one of the most widespread and commonly occurring pathogenic bacteria found in nature, and many of the *E. coli* O157:H7 strains secrete type 2 Shiga toxins. The Shiga toxin secreted by *E. coli* O157:H7 binds to the gut walls and induces cellular death. This can lead to a number of serious complications, including inflammation, ulcers, and even extreme cases of sepsis or meningitis in babies and young children. In this context, quantitative detection of *E. coli* has set a high priority within the field of environmental health, medicine, pharmacy, and food safety [1, 2]. The detection of pathogenic microorganisms was conducted using a variety of strategies, including enzyme-linked immunosorbent assays (ELISAs), polymerase chain reactions (PCRs), quartz crystal microbalance resonators (QCMRs), as well as colony counting. ELISAs and PCRs allow for the detection of the presence of certain molecules associated with the pathogen, while QCMRs measure the mass of particles in a sample, which can indicate the presence of the pathogen. Colony counting is the traditional method of counting the number of colonies of a pathogen on a Petri dish, providing a direct measure of the viable pathogen. The utilization of nucleic acid-primarily based strategies through the application of PCR has become a growing trend owing to their high sensitivity and fast results. Traditional PCR tests tend to have many challenges since they detect both viable cells and dead cells and a complicated standardized protocol would need to be followed in order to detect both. It is possible to predict antibody responses using immunological methods, such as ELISA, which takes approximately 24 hours. Despite the fact that these methods are efficient, they take a significant amount of time, require skilled operators, and are expensive to use. Although technology has advanced greatly over the past couple of decades, conventional techniques remain a crucial tool for detecting and identifying pathogens in raw food, food products, and processing lines. However, rapid microbiological testing has become more popular than traditional microbiological testing in today's world. Conventional time-consuming methods with detection times taking several days are being replaced by rapid tests that take only a few hours [3-6]. Biosensors have been playing an increasingly significant role in rapid detection of bacteria due to their portability, sensitivity, and selectivity [7, 8]. Additionally, on-site biosensors can provide results in minutes on the spot and can easily be deployed in the field [9]. During the last several decades, surface plasmon resonance (SPR) based sensors have grown increasingly popular as a research topic and have been the subject of many papers due to their widespread application in various fields over the years. The SPR technique can be used to measure interactions between molecules in real time without any labeling [10-12]. The application of optical technology in biosensing is prevalent due to its sensitivity, specificity, potential for multiplexing, low sample volume and low noise background. A variety of biomarkers can be detected with optical-based biosensing devices, which are quick, real-time, and label-free, have low detection limits, and are highly sensitive [13-15]. As an optical biosensor, SPR has been extensively used and has been commercialized under a number of different trade names, and is produced by a number of companies all over the world [16, 17]. SPR biosensors utilize receptors that are immobilized on a metal surface, accompanied by a fluidics system that delivers the ligand solution to the sensing layer. The interaction between the ligand and receptor induces a refractive index (RI) alteration, analogous to the effects caused by temperature variations or changes in the bulk refractive index of the surrounding liquid medium. By observing the time-dependent and equilibrium changes in the refractive index, valuable insights into binding kinetics and thermodynamics can be extracted [18].There have been several types of SPR-based biosensors developed for the detection of foodborne pathogens, and researchers have demonstrated the use of these SPR-based biosensors for the detection of pathogens in real food samples [19-29]. In one of the studies, the aim was to detect *E. coli* O157:H7 in pasteurized milk at concentrations ranging from 0 to $10^7$ CFU per mL and it is concluded that the use of SPR biosensors for bacterial detection appears to be a promising method that could be modified to detect multiple bacterial pathogens rapidly, label-free [30]. The presence of *E. coli* O157:H7 in hamburgers and cucumbers was also investigated in another study. The limit of detection for cucumber samples was 57 CFU/mL, while the limit of detection for hamburger samples was 17 CFU/mL [31]. There are many components in food materials, including water, saccharides, lipids, proteins, vitamins, minerals, and low-molecular-weight additives, with various contents, and all of them have a variety of properties. As a result of this complexity of the food sample, it is still challenging to apply a method that can be used for both sample preparation and detection in crude food samples for the purpose of reliable biosensing. [32]. Therefore, it would be desirable to

have a system that could be scaled down to be used as a portable device, while reducing the complexity of the biosensor system, both for the sample preparation and the analysis steps of the process.

Taking this into consideration, the goal of our work is to detect pathogenic microorganisms in complex food matrixes with a high degree of specificity and selectivity, using a SPR-based sensor, which has the advantage of being highly sensitive, affordable, and capable of measuring the changes in resonance angle as analyte bound in a short period of time. We focused on sample preparation prior to detection in order to obtain suitable samples for SPR analysis by minimizing the interfering particles that may interfere with the analysis. The purpose of this study was also to demonstrate a sample preparation protocol that maximizes the recovery of microbial concentration during the detachment step of the process. The SPR biosensor was used to detect *E. coli* in contaminated bay leaves by enabling the sample to be fed into the two different injection gateways simultaneously, which leads to enhanced sensitivity and selectivity in the detection of *E. coli*.

## MATERIALS and METHODS

### Bacteria

*Escherichia. coli* O157:H7 and *Salmonella* Enteritidis were obtained from the Microbiology Lab at the Department of Food Engineering, University of Mersin, and were grown in Tryptic Soy Broth for 24 hours at 37°C. Samples were then prepared by diluting the cultures in PBS (pH 7.4).

### Reagents

The following reagents were obtained from the following sources: A biotin-labeled polyclonal goat anti-*E. coli* antibody was obtained from Thermo Scientific (Rockford, IL, USA); broth and agar plates were obtained from Merck, and phosphate buffered saline (PBS, pH 7.4) and 3-Aminopropyltriethoxysilane (APTES) were obtained from Sigma-Aldrich. It should be noted that all the other reagents were all chemical analytical grades.

### SPR Device

The experiments were carried out using the SPR-Ultimate device developed by Nanodev Scientific, Ankara, Turkey. Our system is described in detail in one of our previous publications and can be found there [33]. There are two different Polytetrafluoroethylene (PTFE) gateways into the flow cell that contain the sensor chip and that is the channel for passing reagents and suspensions containing the target organisms through [34]. It was the microfluidic flow cell that was the first type of injection system that was used. A peristaltic pump is connected to a bonded input and output tubes connected on the chip, so that samples and reagents can flow into the flow cell laminarly at the flow rate of 1.6 µL/s. In contrast to the first type, the second type was injected directly into the flow cell using a micropipette instead of using a tubing system, which allowed real-time detection of results since the samples and reagents were injected directly into the flow cell. All reagents and bacterial solutions were incubated in this port for ten minutes. Henceforth, the first and second methods described above will be referred to as Type 1 and Type 2, respectively (Fig 1).

### Production and Preperation of Au Chip

Among the commonly used materials for biosensor chips, gold is often applied as a metal layer in surface plasmon resonance (SPR) based biosensors. Gold possesses excellent optical properties, facilitating the excitation and detection of surface plasmons, which are responsible for generating the SPR signal.. A method that Ekiz outlined for producing Au coating on SPR chips was used to produce the chips [34]. Assembled chips were cleaned with piranha solution for two minutes, after which they were thoroughly washed with deionized water (DIW) to remove traces of piranha. After the chip was cleaned and dried, it was incubated for at least one hour at room temperature in 3% APTES in ethanol for surface modification [35].

### Preparation of Bacterial Suspension and Inoculation

*Escherichia coli* O157:H7 cultures were grown on Tryptic Soy Broth (TSB) for 24 hours at 37°C. In order to grow the culture to logarithmic phase, one loop from overnight culture was transferred to sterile TSB media. The culture was diluted and adjusted to a concentration of 8 log CFU/mL by measuring turbidity with a spectrophotometer. The inoculum was prepared by centrifuging 10 mL of bacterial culture at 5000 g for 10 minutes after which the cell pellets were washed twice with 10 mL sterile deionized water to remove any residual TSB. The supernatants were discarded, and the pellets were resuspended in 10 mL sterile deionized water. Bay leaves were obtained from Tarsus Mersin, Turkiye. After the leaves were harvested, they were refrigerated until the experiments. As part of the microbial decontamination

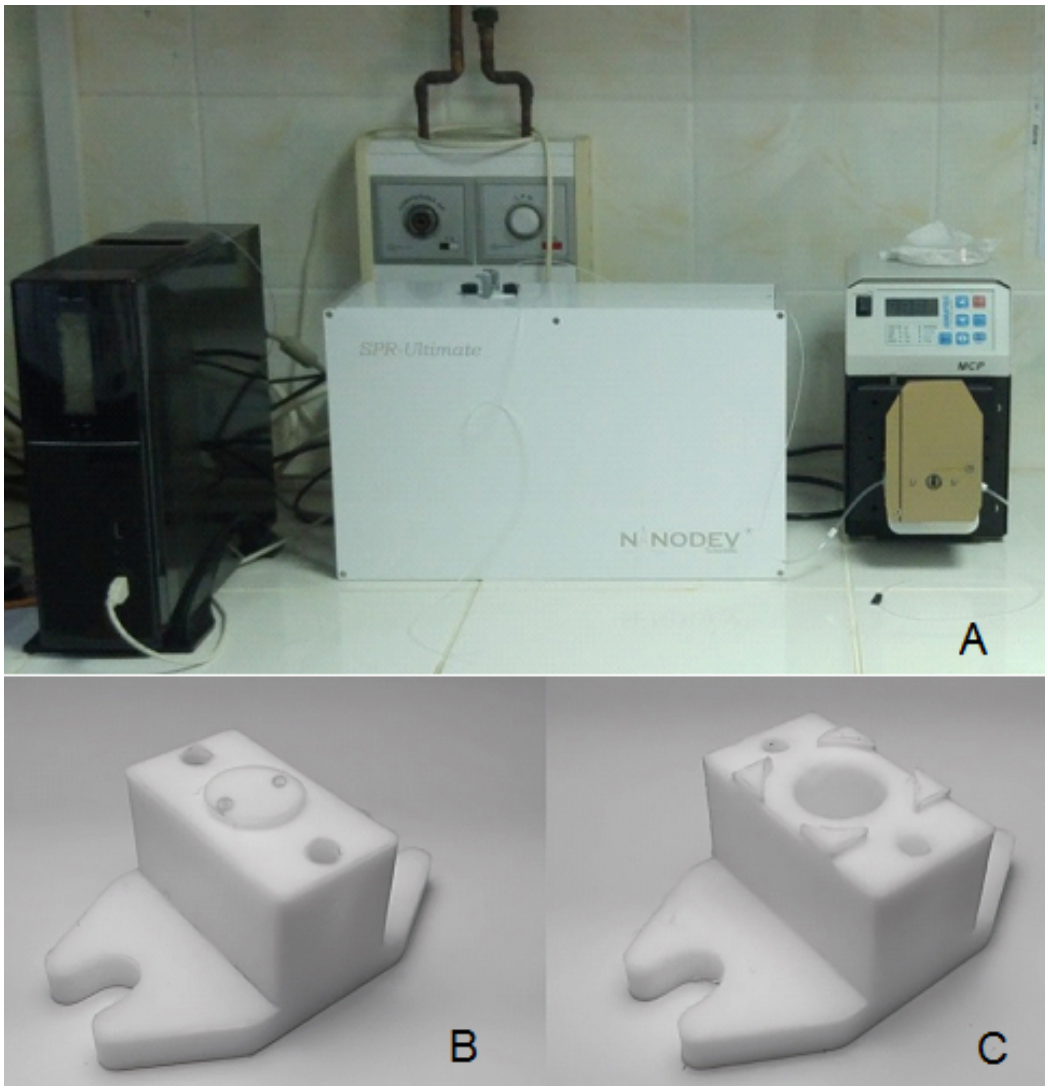


**Figure 1.** Overview of the SPR based sensor system (A). Type 1 (B) and Type 2 (C) injection ports.

procedure, samples were washed with tap water and dipped into 70% ethanol solution for 15 minutes, then rinsed with sterile water and immersed for 10 minutes, then allowed to dry in sterile laminar air flow cabin for one hour. As soon as the samples were dried, each leaf was taken aseptically using a sterile tweezer and placed under a biosafety cabinet on a sterile Petri dish. Sterility of samples was checked for the existence of bacteria after plating on plate count agar (PCA) for an overnight incubation at 37°C. Inoculated samples were prepared by weighing 1 g of bays, spraying them with 1 mL of bacterial suspension, and drying them under aseptic conditions for 60 minutes.

**Bacterial Detachment and Recovery for Plate Counting**

The inoculated samples (1 g) were mixed with 9 mL of phosphate buffer and homogenized for 5 minutes in a stomacher (Interscience Bag Mixer, France) to allow bacteria release. The approximate inoculation level (7.0 log CFU/g leaves) was determined by spreading appropriate dilutions of bacterial suspensions onto PCA agar plates and observing their growth on those plates. The extracts filtered by a stomacher bag filter was centrifuged (5000 g, 10 min) and pellets were washed with PBS and stored at 4°C until SPR analysis. As a further step, bacterial suspensions at concentrations ranging from $10^1$ to $10^7$ CFU/mL were prepared by serially diluting the cultures in PBS.

**Immobilization of Antibodies**

Firstly, the chip surface was washed with deionized water and then a baseline was obtained by injecting PBS onto a clean, gold surface. 38 mg of EDC with 12 mg of NHS was mixed in PBS and allowed the mixture to react for approximately 2 minutes before injecting the sample into the flow loop directly after it had been treated with 20 g/mL of antibody and allowed to react for another 2 minutes. In order to remove excess antibodies, PBS was used as a washing solution. As a means of washing off reversibly bound antibodies from the surface, 0.05 % PBS Tween 20 solution was injected [33] Determination of the optimum concentration of antibody was given more detailed in doctoral thesis of the first author [36].

**Detection of *E. coli* with SPR Sensor**

Starting from low to high concentrations of bacteria, 1 mL of solutions containing bacteria were injected into the flow cell. The unbound antigens were prewashed by passing PBS through a flow cell. During the binding process of bacteria cells to the sensor surface, resonance angle changes were observed as an indication of the binding process. A 0.05 % solution of PBS Tween 20 was injected into the sensor as part of the regeneration process after each analysis. A similar assay procedure for *Salmonella* Enteritidis was followed in order to determine the sensitivity of the sensor developed for *E. coli* detection. Based on resonance angle changes obtained for each bacterium, comparisons are made. The bay leaf samples that were inoculated with *E. coli* were also analyzed using plate counting in parallel with the SPR sensor in order to confirm the results. As part of the plating technique, the samples were serially diluted in saline solution, and 100 µl of dilution was plated on PCA in duplicate and incubated for 24 to 36 hours.

## RESULTS AND DISCUSSION

**Monitoring of the binding between the antibody and the bacteria**

Designing biosensors involves the crucial step of immobilizing biorecognition elements on sensor chips. This process is essential for ensuring the accurate and reliable detection of target analytes. However, simply immobilizing the biorecognition elements is not sufficient; the sensor chip's surface chemistry must be carefully designed and functionalized to optimize the performance of the biosensor. There is a diverse range of surface chemistry techniques available for functionalizing biosensor chips. These techniques aim to modify the surface properties of the chip to enhance the sensitivity and selectivity of the biosensor. For this purpose, self-assembled monolayers (SAMs) encompassing silanes and alkyl phosphates/phosphonates have been employed for the modification of metal oxide surfaces due to their convenient application and commercial accessibility. SAMs can incorporate diverse functional or reactive groups capable of subsequent covalent coupling reactions. Silane-based SAMs facilitate the creation of surfaces with precisely defined topographical features, thereby enhancing bioimmobilization and permitting control over the spatial separation between the sensor surface and the immobilized biomolecule, thereby localizing binding events within the region of highest optical intensity. The compound aminopropyl-triethoxysilane (APTES) serves as a representative SAM and engages with hydroxyl (OH) groups. Generally, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) is employed as a crosslinking agent between APTES and antibodiesis instrumental. Additionally, bovine serum albumin (BSA) plays a dual role, functioning as a cross-linker between the substrate and proteins, while also acting as a blocker to inhibit nonspecific protein adsorption [37]. The initial endeavors concerning antibody immobilization onto glass surfaces aimed at their direct covalent attachment. This approach demonstrated the potential for creating stable surfaces with antibodies arranged in a specific orientation, relying on unique surface moieties available for modification. However, this method was observed to yield diminished activity for the immobilized biomolecules in certain cases. Multiple distinct strategies were initiated, commencing with glass surfaces functionalized using amine groups. To this end, the aminosilane compound, 3-Aminopropyltriethoxysilane (APTES), was covalently linked through silane chemistry to produce a uniform functional surface which is tightly attached to the chip surface . Before the covalent attachment of *E. coli* cells on chip surface, which were firstly immersed in %3 (v/v) APTES solution for 1 h to introduce glass slide surfaces amine groups. Following this, the activation of amine groups on glass slides were treated with PBS buffer. Then, cultured live *E. coli* cells were dropped and coated on the activated surfaces. During this study, the first objective was to immobilize anti-*E. coli* polyclonal antibody onto the gold sensor surface for the purpose of rapid detection of *E. coli*. Therefore, we investigated how resonance angle values changed during antibody immobilization. The resonance angle of the antigen-antibody interaction increased

during the binding process. Following the washing with PBS it was found that the signal was significantly decreased due to the removal of weakly bound antibodies from the surface. Anti-*E. coli* immobilized to the surface still led to a significant change in angle, indicating good immobilization. As soon as the antibody had been immobilized, diluted bacteria samples in PBS were injected onto the sensor surface with various concentrations of antibodies (ranging in concentration from low to high concentration) for binding to the immobilized antibodies in order to detect *E. coli*. Fig. 2 shows a schematic diagram of the immobilization process for anti-*E. coli* as well as all dilutions of bacteria prepared from pure cultures that are used for immobilization. Furthermore, the variations in resonance angles that occurred during the feeding of bacterial dilutions to the system have been analyzed and shown in a more detailed manner in Figure 3, which illustrates the binding of *E. coli* with increasing concentrations. In each step, once the surface had been saturated with the bacteria, the loosely bound ones were washed with PBS and the change in resonance angle was determined by taking the difference between the first signal from the PBS solution (baseline) and the signal from each concentration of bacteria. Each concentration was calculated in accordance with this method in order to determine the average change in resonance angle. Accordingly, these values were used to create the calibration curve that can be seen in Figure 4. The resonance angle was observed to increase continuously as the concentration of the bacteria increased. A good correlation existed between the microbial load and the change in the resonance angle of a sample for each dilution, with the value of R-squared as 0.97459. Based on the relationship between the resonance angle value and the logarithm of the bacteria concentration, it becomes possible to determine the bacterial concentration in an unknown test sample. Similarly, Dudak and Boyacı studied the rapid, sensitive and selective detection of *Escherichia coli* using a SPR based sensor, and a linear correlation of bacteria concentration and change in response unit was found with an $R^2$ value of 0.976 [38].

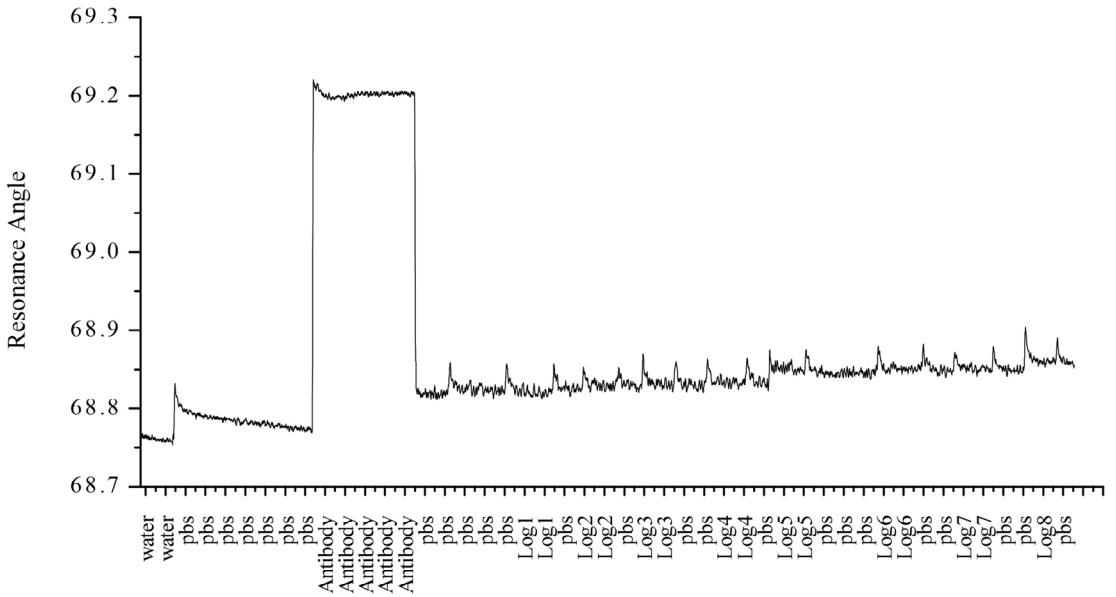


**Figure 2.** The changes in resonance angle during the binding of antibody and serially diluted Escherichia coli suspensions to the sensor surface.

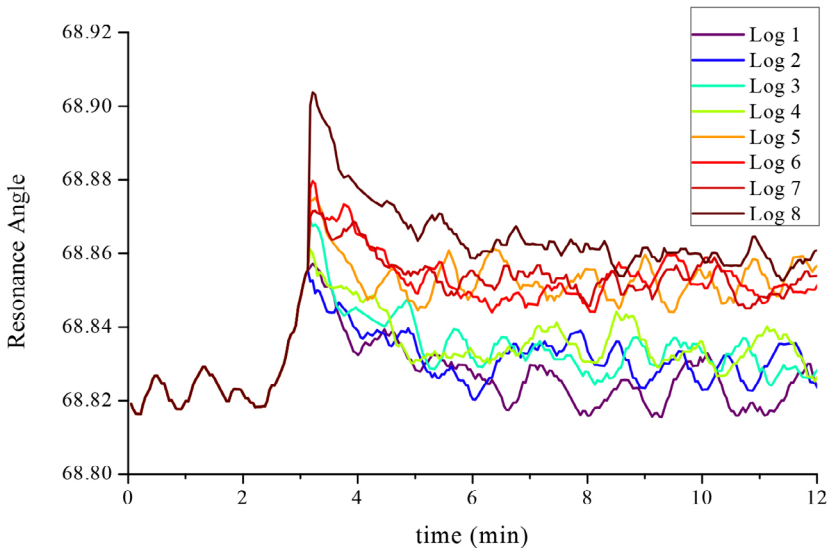


**Figure 3.** The change in the resonance angle with the binding of *Escherichia coli* at concentrations between log 1 and log 8.

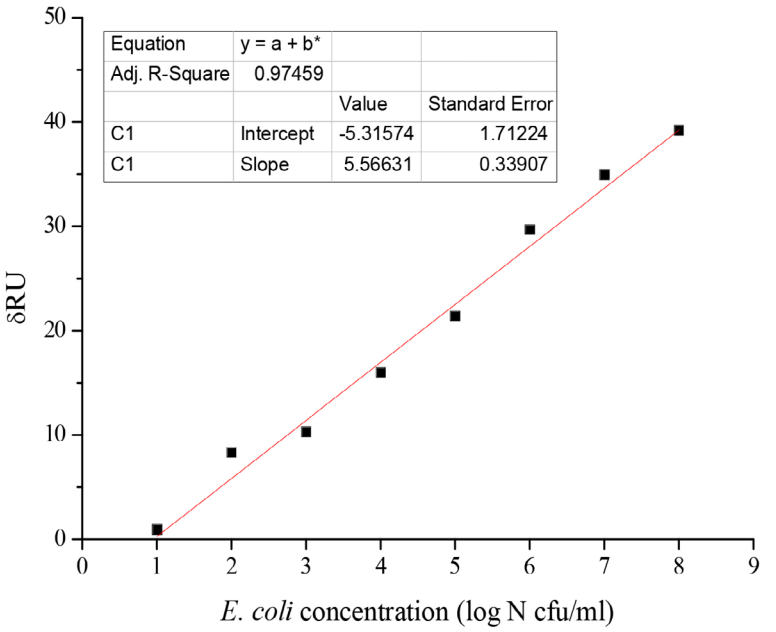


**Figure 4.** The lineer relationship between the change in the response unit and concentrations of *Escherichia coli*.

**The performance of the sensor for detecting *E. coli* O157:H7**

There are several methods available in the literature that can be used to estimate the limit of detection and quantitation. A common method used in many studies to calculate the detection limit was to multiply the noise three times. Nevertheless, Shrivastava and Gupta have defined the Limit of Detection (LOD) and Limit of Quantification (LOQ) values in a different manner, as outlined below [39]:

$$\text{Detection/Quantification Level} = \frac{F \times SD}{b}$$

Where ;
Where

F: The factor for DL and QL is 3.3 and 10, respectively.

SD: A standard deviation can be calculated from standard deviations of the blank, ordinate intercept, or residual standard deviations in a linear regression.

b: Slope of the regression line.

PBS with a concentration of 10 mM was used as a stock solution during our experiments as a buffer solution. Stock buffer was diluted to the 5, 2.5, and 1.25 mM levels in order to be fed into the SPR system for calculation of the LOD and LOQ values [40]. Signals were obtained and are shown in Figure 5 as a result of the measurements. Based on Shrivastava and Gupta's approach, the limit of detection and limit of quantification values were calculated to be 5.2683 and 15.9645, respectively. Based on this data, it can be concluded that it is possible to detect *E. coli* with a quantification limit of 10000 bacteria. Smilarly, a lectin-based SPR biosensor was used by Wang et al. for the detection of *Escherichia coli* O157:H7 in food samples using a lectin-based SPR biosensor. According to them, the control signal was measured to be 7 RU and the signal of the bacterial dilution containing 3 log CFU per mL was measured to be 26 RU, which was three times larger than the control signal. This result indicates that the limit of detection of the lectin-based SPR biosensor is 3 log CFU/mL[41].

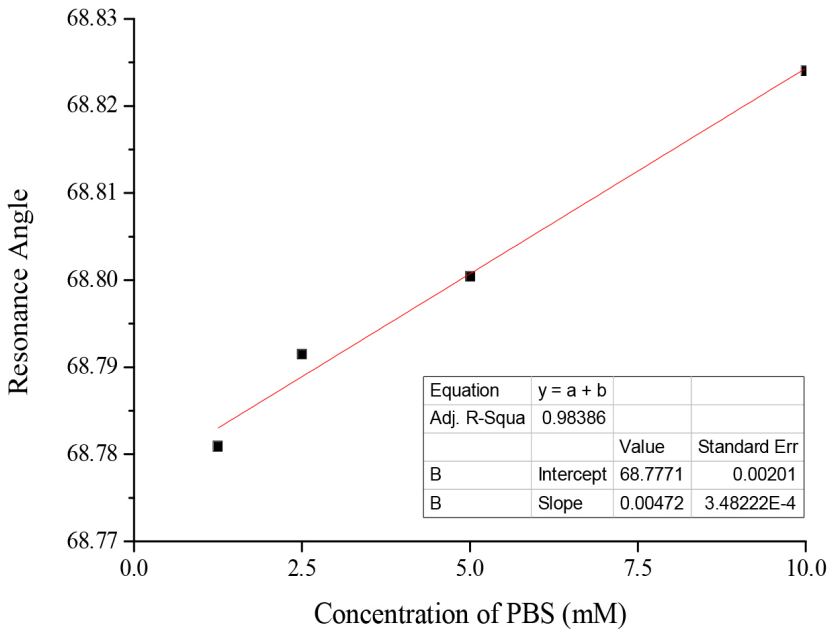


**Figure 5.** SPR signals of different concentration of PBS (1.25, 2.5, 5 and 10 mM) in DIW.

**Specificity Testing**

The change in RU after the binding of *Salmonella* Enteritidis ($10^7$ CFU/mL) on the biosensor was measured in order to investigate the selectivity of the biosensor in comparison with *Escherichia coli* instead of *Salmonella* Enteritidis ($10^7$ CFU/mL). For equal concentrations of *Salmonella* and *E. coli*, the change in the response was 5.43 RU and 49.76 RU, respectively. It is important to note that the response to the non-specific binding was much lower than the response to *E. coli* which shows how specific the biosensor is.

A similar study was conducted by Wang et al. to test the selectivity of lectin-based SPR biosensors against both target (*E. coli* O157:H7) and non-target (*Salmonella* Enteritidis) bacteria. The SPR response values were classified into three ranges and the researchers found that this biosensor exhibited a high level of specificity for the detection of *E. coli* O157:H7 [41].

***E. coli* O157:H7 detection in bay leaves**

Microbiological analysis plays a critical role in ensuring the safety and quality of food products. The choice of sample matrix in microbiological analysis has significant implications for the accuracy and reliability of the results. The complexity of high-fat and high-protein foods necessitates additional sample preparation steps, such as homogenization, enrichment, or filtration, to extract microorganisms from the matrix effectively. These steps can be time-consuming, labor-intensive, and may require specialized equipment. In contrast, the use of simple food materials reduces interferences, enhances specificity, simplifies sample preparation. In order to overcome these disadvantages, we have chosen to study a relatively simple, but potentially microbiologically hazardous herb-spice from the laurel leaf group of plants, namely bay leaf. Several outbreaks of *Escherichia coli* O157:H7 infections associated with leafy greens have been reported in the literature in the past. Using this bioselectisensor system based on SPR, the bacterial load was measured in a selected real food sample as well. Bay leaf was chosen as a good and easy material for bacterial inoculation and recovery procedures since it has a smooth and sufficiently wide surface that is suitable for bacterial inoculation and recovery procedures. The sensor was used to analyze samples inoculated with *E. coli* O157:H7 diluted from $10^1$ to $10^7$ CFU/mL, in order to find the changes in resonance angle due to different injection systems. The data were collected by sampling for ten minutes for each dilution, followed by recording by the software a report on the results. According to Figures 6 and 7, you can see how *E. coli* could be detected in bay leaf samples divided into Type 1 and Type 2 injection systems. There was a relationship between the changes in resonance angles and the changes in microbial load, and this relationship is nicely represented in Figure 8.

As a result of the measurement, the values of resonance angles did not change significantly in the dilutions containing low levels of bacteria during the experiment. Interestingly, the two methods did not exhibit the same trends when it came to the changes in response. The flow rate of the fluid was seen to be the cause of this difference [42, 43]. Flow rate is one of the most important variables that will determine the sensitivity of a SPR device and will have a direct impact on the angle of resonance as well. In a flow cell, pressure changes as a function of flow speed [34]. It was apparent that after dilution with 4 log CFU/mL of Type 1 solution, a dramatic increase in SPR signal was observed . In contrast to Type 1, the resonance angle of Type 2 was gradually increased in comparison to Type 1. As the bacteria load increased with the fourth dilution, a continuous increase in the resonance angle was observed with the increasing concentration of bacteria. As it is evident; flow rate plays a crucial role in binding events of SPR-based biosensors. High flow rates are essential for kinetic experiments to minimize mass transport effects. In steady-state experiments, higher flow rates are not necessary as long as a clear steady-state plateau is achieved for determining RU at the steady-state. Optimum flow rate may vary for each system and requires experimentation. Detailed information about how the optimal flow rate was determined can be found in my doctoral thesis, and it can be seen that the flow rate of 1.6 µL/s was selected because of the mathematically minimal error margin it is likely to have [36]. Consequently, bacterial cells were successfully detected at low levels and it was found that the SPR-based sensor could be applied to both pure culture and selected food materials for the rapid detection of their microbiological characteristics. As part of a sensitive analysis, it is fairly important to use a suitable preparation technique in order to resolve the species of bacteria and remove most of the interfering factors from the food matrix while performing the analysis. There is still room for further improvements and optimization of the sample preparation process in order to obtain a more accurate result when we are dealing with more complex food samples.

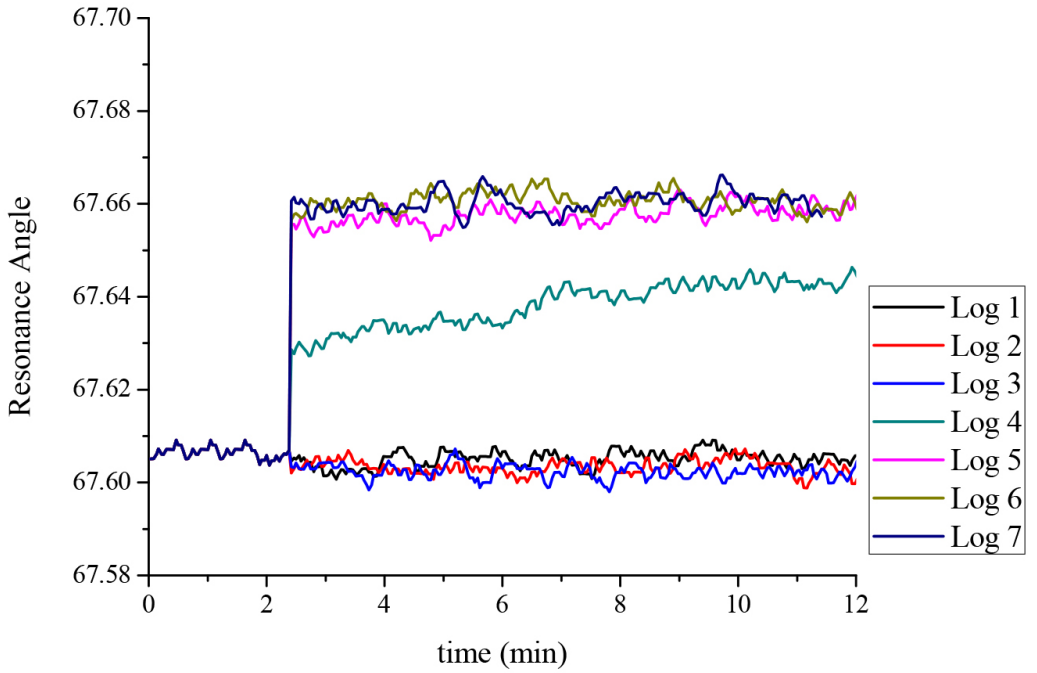


**Figure 6.** The trend of the measured resonance angle for each concentration of *E. coli* with Type 1 injection. Increasing the bacterial concentration increases the resonance angle.

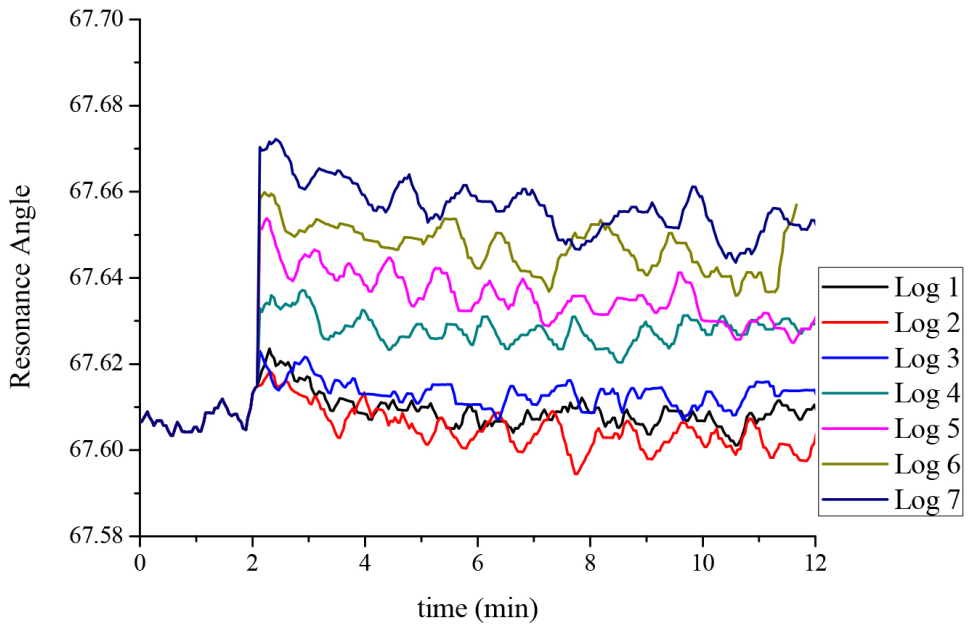


**Figure 7.** The trend of the measured resonance angle for each concentration of *E. coli* with Type 2 *injection*. Increasing the bacterial concentration increases the resonance angle.

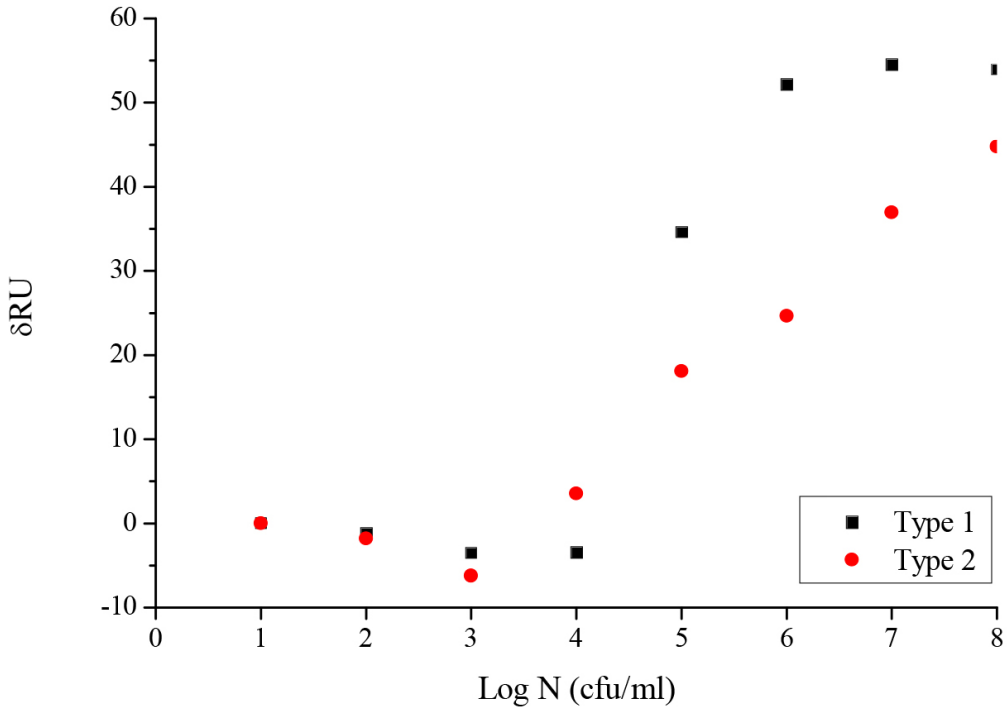


**Figure 8.** The relationship between the change in the response unit and concentration of Escherichia coli recovered from bay leaves.

We found that our SPR sensor system was capable of detecting an array of low levels of bacteria, and that this detection limit was comparable with that of other methods in the literature. It has previously been reported that a SPR based biosensor was evaluated for detection of *E. coli* O157:H7 in apple juice, pasteurized milk, and ground beef extracts. The bacterial concentration was determined using a LOD range of about $10^2$–$10^3$ CFU/mL by taking three times the standard deviation values from the negative control as a measure of bacterial concentration. [30]. When monoclonal antibodies at concentrations ranging from 5 to 50 mg/mL were immobilized to the sensor surface, it has also been demonstrated that the detection limit for *E. coli* O157:H7 was between $10^6$ and $10^8$ cells per mL using SPR based biosensors [24]. The rapid detection of *Escherichia coli* O157:H7 using SPR has been described by Si et al and the limit of detection was found to be 3 x $10^5$ CFU/mL and the change of RU was found to be correlated with the concentration of *E. coli* O157:H7, and the $R^2$ value was found quite high (0.99). The authors of the study recommended this method as a convenient and stable method to be applied to food areas [44]. SPR biosensors were compared with ELISA and an order-of-magnitude reduction (from 3.0 × $10^4$to 3.0 × $10^5$ CFU/mL) on detection limit was investigated by Wang et al. [45]. In the same way, Meeusen et al. have determined that the limit of detection for *E. coli* O157:H7 is 8.7 x $10^6$ CFU/mL. [46]. *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and *Listeria monocytogenes* were detected simultaneously by a multichannel SPR device designed by Zhang et al. In accordance with the conclusions of the study, the detection limits were determined to be 0.6 × $10^6$, 1.8 × $10^6$, and 0.7 × $10^7$ CFU/mL for *E. coli, S.* Enteritidis, and *L. monocytogenes*, respectively [47]. It was also reported by Zhang et al. in another study as to how seven pretreatment methods affected the accuracy of *E. coli* O157:H7 detection by SPR Biosensor using seven pretreatment methods, and they found the detection limits to be between l$0^5$ and $10^8$ CFU/mL level depending on the method used. In their paper, the authors recommended the use of sonication for the preparation of samples in order to detect the presence of *E. coli* O157:H7 using an SPR based biosensor [48]. There are also several review articles present the recent advances in different optical sensing techniques for the pathogen detection especially for *E. coli* [49].

Comparatively with the SPR-based biosensors cited above, our device has a lower complexity than several other commercially available devices that have been marketed under different trade names. Additionally, for a biosensor to be effective, it needs to be affordable, portable, and easy to use for it to meet the above requirements. This study yielded highly satisfactory results relating to low detection limit, but the most prominent outcome of the work is the application of the sensor for the direct assessment of pathogens isolated from food materials. There is a need for more studies designed to improve sample preparation and analysis steps to detect pathogens in low concentrations, in order to be able to analyze complex food samples reliably, rapidly, accurately, simply, sensitively, and selectively.

## Conclusion

A SPR-based biosensor can be used to ensure the safety of the food supply chain, since it is easy to use, portable, reagentless, and provides results within minutes. There has been suggestions from the results that it may be possible to use rapid sample preparation and direct detection of bacteria directly from complex food matrices for application in real-time screening in food production lines to allow real-time identification of bacteria. Detection of bacteria in serially diluted suspensions can be accomplished with the SPR based biosensor owing to its lower detection and quantification limits. In the present study, the biosensor was able to differentiate between cellular concentrations between $10^3$ and $10^7$ CFU/mL and showed promising results in detecting various pathogens in various food samples. The most significant aspect that makes this study innovative is the utilization of two different injection systems. In one of the systems (Type 1), the attachment of molecules onto the chip is investigated under a continuously steady flow rate, while in the other configuration (Type 2), a specific volume of sample, is transferred on the chip form of a hole using a pipette without any flow rate and establishes a static environment. Thus, both scenarios are comparatively examined, investigating the impact of flow rate presence or absence on the binding dynamics. In order to ensure food safety, the ability for any detection method to detect lower levels of pathogen contamination in a food system is crucial. When a low level of contamination is expected, the current protocol would need to be supplemented with an enrichment procedure to ensure an adequate level of contamination. Most types of biosensors are still limited in their ability to identify the lower levels, so the biosensor may be used in the future to monitor bacterial growth in real time and detect lower levels of the pathogen using a temperature controlled incubation port that is combined with the system as an enrichment method. Future research will therefore focus on improving antibody immobilization and the sensitivity of the bacterial detection method, which will ultimately result in a greater level of sensitivity and a lower limit of detection.

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