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The Determination of *Listeria monocytogenes* in Foods with Optical Biosensors

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ABSTRACT *Listeria monocytogenes* is a food-borne intracellular pathogen that is resistant to adverse conditions of food processing and is a causative agent of high mortality listeriosis disease. Analysis of large food lots is limited by culture methods. Nowadays, although the conventional culture methods have been adopted as the gold standard method, they can be insufficient to control food parties that reach very large quantities. Biosensors with on-site detection potential and their characterizing with high accuracy and precision offers many opportunities for real-time estimation of industrial contamination. As the most promising technique for rapid detection of *L.monocytogenes*, ones based on surface plasmon resonance (SPR) and fiber optic biosensors (FOBs) where direct or indirect detections can be made by detecting the interaction of the biomarker element with the optical field without marking or labeling, have come to the fore. SPR and FOBs can be detected directly in the range of 10^2-10^6 cfu/ml and ≤10^1-10^3 cfu/ml, respectively.

Keywords: *Listeria monocytogenes, Biosensor, Surface plasmon resonance, Fiber optic*

ÖZ

Gıdalardan *Listeria monocytogenes*'in Optik Biyosensörlerle Belirlenmesi

Listeria monocytogenes gıda kaynaklı intrasellüler patojen olarak gıda işlemenin olumsuz koşullarına dayanıklı ve yüksek mortaliteli listerioz hastalığının etkenidir. Büyük gıda partilerinin kültür yöntemleri ile analizi sınırlıdır. Günümüzde, geleneksel kültür yöntemleri altın standart yöntem olarak benimsenmesine karşın, çok büyük miktarlara ulaşan gıda partilerini kontrol etmede yetersiz olabilirler. Biyosensörlerin yerinde tespit potansiyeli ile yüksek doğruluk ve hassasiyetle karakterize edilmeleri, endüstriyel kontaminasyonları gerçek zamanlı tahminde birçok fırsat sunmaktadır. *Listeria monocytogenes,* hızlı tespitte en umut verici teknik olarak işaretleme veya etiketleme yapılmadan optiksel alanla biyotanıyıcı eleman etkileşiminin optik sinyallerle belirlenerek doğrudan veya endirekt tespitlerin yapılabildiği yüzey plazmon rezonans (SPR) ve fiber optik biyosensör (FOB) esaslı olanları ön plana çıkmıştır. SPR ve FO biyosensörleri ile sırasıyla 10^2-10^6 kob/ml ve ≤10^1-10^3 kob/ml aralıklarında doğrudan tespit yapılabilmektedir.

Anahtar Kelimeler: *Listeria monocytogenes, Biyosensör, Yüzey plazmon rezonans, Fiber optik*

INTRODUCTION

Listeria monocytogenes is defined as a food-borne pathogen and as causative agent of listeriosis disease with low morbidity and high mortality. The pathogen can survive under most of adverse conditions of food processing stages and cause contamination. In many countries, the restrictions are imposed to food products, especially the number of ready-to-use products.

Nowadays, although the conventional culture methods have been adopted as the gold standard method, they can be insufficient to control food parties that reach very large quantities. Precise, fast and reliable methods come to the fore. There are many quick detection methods based on different principles.

In addition to providing rapidly and directly to obtain the results, biosensors also have the potential to provide the on-site detection of the, which allows rapid monitoring of important or critical control points in food processing. The high accuracy and sensitivity of biosensing techniques have made it the key to increasing biosensor applications in food quality control.

It is possible to classify biosensors used in foodborne pathogen and toxin detection as optical, electrochemical and piezoelectric biosensors on the basis of transduction mechanisms. Most of the biosensors used in the food industry and research laboratories are optical ones. In this review, we will investigate the use and the advantage of optically based biosensors in the detection of *L.monocytogenes* in foods.

Listeria monocytogenes

L. monocytogenes was firstly isolated from a human with meningitis in France in 1921, then from other mammalian and non-mammalian species. It was not identified as the

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causative agent of food-borne outbreaks in humans until the 1980s (Walland et al. 2015).

L. monocytogenes, which is common in nature, can grow at refrigerator temperature, may contaminate many foods during production, packaging, transportation and storage, is a food pathogen, becoming an important public health subject with consumption such as contaminated foods because of causing severe and nonenteric features of listeriosis in humans as intracellular pathogen (Mor-Mur and Yuste 2010; Sireli and Erol 1999). The bacterium is Gram positive, facultative anaerobe, oxidase negative, catalase positive, small moving shaped-rods (0.4-0.5×1-2 µm) and non-spore. It is movable at 22-30 ˚C and is immobilized at 37 ˚C due to its inability to develop flagellum. In addition it can develop in adverse environmental conditions such as high salt concentration, low temperature (development at 40.4 ˚C), high temperature (development at maximum 45 ˚C), a wide pH range (4.4-9.4) and low water activity (as like 0.9) . The pathogen is completely inactivated at 75˚C (Chlebicz and Slizewska 2018).

L. monocytogenes is characterized by high genetic differences. In other words, it contains numerous strains. There are reports of differences between strains related to virulence and contamination (Walland et al. 2015). The serotyping developed for subtype differentiation revealed that the majority of human and animal infections were caused by only three different serotypes (1/2a, 1/2b and 4b). These serotypes are also the most common serotypes isolated from food (Walland et al. 2015).

The presentation of the intracellular pathogenicity of *L. monocytogenes* in host organisms means the infection. The pathogen has the ability to penetrate not only the intestinal cells, but also the spleen, liver, brain, heart and placenta. It settles in the vacuole after cell invasion. The pathogen separated from this location begins proliferation, and is activated in other cells by the actin-based motility mechanism without stimulating the immune response of the host organism. The pathogenicity is due to a number of virulence factors such as the adhesive protein (LAP), internalin, listeriolysin O, phospholipase C, actin polymerization protein (ActaA), which is the superficial protein, and OrfX protein (Chlebicz and Slizewska 2018).

1. Biosensors

The detection of *L. monocytogenes* is primarily important for the food industry, water and environmental quality control and public health. In technologies where biosensors are used, biological receptors are physically or chemically coupled to converter called as the transducers, thereby yielding unique tools with great potential for rapid, online and real-time diagnosis of biological agents. The basic model of its use is the determination of pathogens from food and environmental sources (Soni et al. 2018).

Biosensors are defined as analytical devices that combine the recognition molecule biologically derived such as antibody, phage, aptamer, single-stranded DNA with a suitable physicochemical transduction mechanism (Iqbal et al. 2000). In other words, these devices from consist of a biomolecule (eg, tissue, microorganism, cell receptors, organelles, antibodies, nucleic acids, enzymes, etc.), that interacts with the targeted analyte in the analyzed sample and is indicated by signals generated the presence of the analyte, a biologically derived molecule (eg, engineered proteins, recombinant antibodies, aptamers, etc.) or biomimic molecule (eg combinatorial receptors, synthetic catalysts, imprinted polymers (Soni et al. 2018).

Biosensors generate an electronic or optical signal that can be measured and recorded in proportion to the specific biological interaction between the analyte and the recognition molecule. With these signals, a wide variety of targets can be detected from small molecular weight proteins to pathogens. If the sensitivity and selectivity of the biosensor is very high, the faster results can be achieved as an alternative to the culture method in practical field applications. In comparison with traditional analysis methods, the biosensor is a device capable of detecting pathogenic antigens and does not require highly trained personnel (Turner 2000).

Biosensors are desired to have many features such as accurate, close to real time, sensitive, specific, reproducible, robust and easy to use. In order to be acceptable practical devices, the number of false positive and false negative results should be very low and zero if possible. Since the most important characteristics of the traditional culture methods are the analysis period, this period should be less than one hour. Sensitive sensors are a must for the food industry. Any false negative results can result in high cost collection and loss of community trust. On the other hand, the false positive result will lead to cost increases that are reflected to the producer and ultimately to the consumer. It is desirable that responses from biosensors be quantitative and reproducible. It must be mechanically and biochemically resistant and robust. Ultimately, the biosensors to be used in the analysis should carry the easiness that does not require the need for highly trained and qualified personnel.

To date, the various biosensors have been developed for the detection of *L. monocytogenes*. Recently, the other many biosensor devices that use piezoelectric, thermometric, micromechanical, cell based and electrochemical transduction elements, and especially optical ones have been extensively investigated for rapid detection (Iqbal et al. 2000; Soni et al. 2018).

In this study 200 food handlers from different plants were interviewed. Questionnaires were responded individually, in the presence of the researcher, 30 minutes time required for completion.

2. Optical biosensors

Optical biosensors (OBSs) are analytical devices capable of detecting interaction the biorecognition between the optical field. Thanks to the transducers used, it is possible to detect bacteria directly without marking or labeling. They can even detect small changes in refractive index and thickness resulting from binding of cells to receptors fixed to the transducer surface.

Optical sensor signals are multi-purpose signals because it is possible to use in the pathogen detection directly or by increasing their intensity with other techniques. OBSs are subcategorized according to their reflection, refraction, resonance, dispersion, infrared absorption, Raman scattering, chemiluminescence, fluorescence. Unlike the others, in fluorescent sensors, a fluorescent compound conjugated to the antibody assists in the detection of the pathogen (Arora et al. 2011). The most commonly used marker routinely is fluorescein isothiocyanate. It is reported that some lanthanides can also be used for the same purpose (Arora et al. 2011).

Currently, fiber optic biosensors (FOBs) are developed for the detection *Listeria* spp. and other pathogens as *E. coli* O157: H7, *Salmonella* spp., botulinum and staphylococcal enterotoxin. (Arora et al. 2011). It has been reported that OBSs are commonly used in surface plasmon resonance

(SPR) and evanescent FOB techniques. (Lee et al. 1997; Turner 2000).

2.1. Surface plasmon resonance based biosensors

Surface plasmon resonance (SPR) biosensor technology is one of the most promising methods for rapid pathogen identification (Joung et al. 2007). There is an increase in the use of SPR-based OBSs that are close to real-time and allow detection without labeling. With these sensors, pathogen detection in the food chain can be made directly and indirectly. (Bergwerff and Van Knapen 2006). Monoclonal or polyclonal antibodies identified to the target pathogen facilitated the direct detection. In pathogen isolation from food, antibodies are immobilized to the solid phase of the sensor. Antibodies can be used in inhibition-like analysis. Incubation of antibodies with the target pathogen prior to detection of unbound antibodies is envisaged. The free antibodies are screened on the surface of an SPR sensor coated with either purified antigens or Fc or Fab binding antibodies. Diagnosis can be made by SPR analysis on the polymerase chain reaction products of toxin genes of pathogens. On the other hand, the possibility of using various metabolic products as biomarkers has provided the possibility of indirect detection with SPR biosensors developed (Bergwerff and Van Knapen 2006).

In the SPR technique, when the target analyte binds to the ligand immobilized to the transducer surface, the OBS detects signals where are produced near the top surface of the sensor, which result from small changes of several hundred nanometers in the refractive index (Joung et al. 2007;Rich and Myszka 2001). SPR biosensors have been developed at wide ranges for the direct detection of *L. monocytogenes* and other food pathogens. The detection range was reduced to 10^2-10^7 bacteria/ml with developed sensors at very low levels depending on the type of detection (Joung et al. 2007).

SPR is defined as an optical event in which resonance is generated by surface plasmon waves, known as excitation and increase in density of electrons by polarized light. The structure of an SPR sensor is formed by a generally gold metal covering electrically insulating material such as quartz. The wave area, which is known as the evanescence wave (EW) field (EWF) and whose amplitude decreases, is formed between the metal and the insulating material and spreads to the external environment. Since EW are exponentially reduced from the surface, surface interactions can be detected by SPR technique (Dover et al. 2009;Shankaran et al. 2007). When SPR occurs, there is a significant reduction in reflected light intensity. The wavelength or angle at which resonance occurs is dependent on the refractive index (RI) of the other insulating layer adjacent the metal layer. Resonance deviations can be determined by monitoring changes in reflected angle, wavelength or reflection intensity. The RI of interface can be calculated from the resonance angle or wavelength (Tubb et al. 1997).

The Krestchmann figuration, which consists of a metal layer placed on the base of the quartz prism, is popular for the usage in surface plasmon resonance devices, and the devices produced on the basis of this figuration are commercially available. Koubov et al. (2001) were able to detect *L. monocytogenes* at 10^6 cfu/ml by SPR technique based on the placement of an optical sensor in the classical Kretschmann prism figuration. Leonard et al. (2004), in the indirect detection of *L. monocytogenes* in buffered solution by SPR technique using BIA3000 detector, firstly incubated *L. monocytogenes* with polyclonal rabbit antibodies and then removed dead bacteria and unbound antibodies from the solution medium by centrifugation and determined the amount of antibody, could not form antigen-antibody complex from the supernatant, by biosensor they developed. They reported that the pathogen had been detected as 10^5 cfu/ml .

In the quantitative and simultaneous detection study of four different pathogen bacteria with an eight-channel optical SPR sensor, it reported that the detection at the level of approximately $3.5x10^3$ cfu/ml may be obtained by complexing biotinylated rabbit polyclonal antibodies immobilized on the sensor surface with *L. monocytogenes* which prepared in phosphate buffered saline (PBS) at a concentration of $5x10^5$ cfu/ml (Taylor et al. 2006). Nanduri et al. (2007) detected the pathogen at $2x10^6$ cfu/ml by applying the biosensor, which they developed by using the single-chain scFv antibody expressed from the surface of Lm P4: A8 coded phage specific ActA, to the SPR technique.

Poltronieri et al. (2009) detected *L. monocytogenes* at 10^2-10^6 cfu/ml in the SPR technique by using an immunosensor consisting of secondary antibodies fixed to the gold electrode. With this study, it was concluded that the use of SPR sensor in water, vegetable and environmental samples would be appropriate. In a study using phage endolysins, firstly affinity levels of endolysins were determined by using SPR technique in pico and nanomolar ratios, then endolysins were labeled with fluorescent marker dyes of various colors. Thereafter, multiplex determinations could be made both species and serotype levels with fluorescence microscope in 15 minutes after they were incubated with milk and cheese samples with inoculated *Listeria* spp. (Schmelcher et al. 2010).

In a study which a gold-plated microchip biosensor was designed for the detection of *L. monocytogenes* and different pathogens by diffraction-network coupled surface plasmon resonance imaging, known as combining the parallel light with surface plasmons, two different monoclonal anti-*Listeria* antibodies and one rabbit polyclonal anti-*Listeria* antibody and the negative control antibodies were immobilized on microchips at equivalent concentrations and analyzed with a sample containing *L. monocytogenes* heat-killed at a concentration of $1.65x10^9$ cfu/ml in PBS with 0.5% Tween 20. It has been found LZF monoclonal antibody caused deviation with a much larger resonance angle than LZH1 polyclonal antibody and reported that the slight differences in the affinity for *L. monocytogenes* of the antibodies may be due to the procedure used to kill the pathogen (Marusov et al. 2012). Together this study, the single-use chips were opened and compact microarray sensoric chips were equipped with antibodies or other specific recognition molecules for multiplex analyte detection. With these chips, the listerias can be detected at a detection limit of 10^6 cfu/ml compared to other pathogens.

In a study conducted with resonance mirror SPR biosensor (IAsys) produced by imbolization of MAb-C11E9 monoclonal antibody, which used in immunoseparation and immunosensor applications, to carboxylate cuvette, live *L. monocytogenes* V7 bacteria were detected in high concentrations such as $1x10^8$, $2.5x10^8$ and $5x10^8$ cfu/ml (Lathrop et al. 2003). In this study, the lack of signal from the IAsys sensor was related to the sensing of EWF at 200-300 nm from the surface. However, in the same study, it was reported that raw surface proteins extracted from *L. monocytogenes* could receive signals. In a study using anti-InlA monoclonal antibodies with code mAb2B3 prepared

for the serotype of *L. monocytogenes* 1/2a on a platform based on SPR and ELISA, it was reported that virulent *L. monocytogenes* could be detected at concentrations of 10^7 cfu/ml (Hearty et al. 2006).

In a study aimed at the rapid and simultaneous detection of *L. monocytogenes* and three food pathogens with surface plasmon resonance (SPR) biosensors, by analyzing the homogenates prepared from sonication of suspensions of different concentrations of pathogens using with SPR biosensors and sensor chips produced by polyclonal antibodies spesific to the pathogens, it was concluded that the working with the precipitates obtained from the centrifugation of homogenates increased the detection sensitivity and reported that the lower detection limit of *L. monocytogenes* may be 0.7×10^7 cfu/ml if the detection from precipitates with a multichannel SPR biosensor is carried out, even if non-target pathogens are at concentrations of 10^5-10^8 cfu/ml (Zhang et al. 2014).

2.2. Fiber optic based biosensors

Fiber optic-based biosensors (FOBs) are produced from tapered silica or polystyrene microfiber material for better transmission and reception of light signals, and react responsively to EWF changes resulting from RI exchange when analyte is bound (Arora et al. 2011; Sharma and Mutharasan 2013; Soni et al. 2018).

FOBs are defined as a sandwich immunoassay based on collecting target pathogens on a polystyrene guide wave with a pathogen-specific fluorophore-tagged biotransmission molecule including antibodies, aptamers and receptor proteins. FOBs can be used directly in preenriched/selectively enriched samples in culture media and results can be obtained within 8-24 hours depending on pathogen type and assay sensitivity. *L.monocytogenes* and many other food pathogens can be used successfully for identification. The pathogen counts can also be done since the signal intensity is proportional to the concentration (Abdelhaseib et al. 2016).

If the sensing surface of fiber is prepared with recognition biomolecules, the target analytes such as pathogen or toxin bind to this fluorescent labeled surface. When binding occurs, they are excited by laser wave (635 nm). The fluorescent signals being by stimulating are detectable by a fluorescent detector. If the biomolecule is antibody, binding can be achieved by avidin-biotin interaction or by simple adsorption. Such binding can be used as a sandwich immunoassay system (Arora et al. 2011; Sharma and Mutharasan 2013; Soni et al. 2018).

When the target analyte is binding to the optical fiber, the changes in optical yield are observed, surface RI modulation occurs as an indicator of EWF change. The fibers operate with minimal loss of light due to the total internal reflection of the transmitted or passed light in the fiber core. The emitted light has two components, which are the core guiding field and exponentially decreasing EWF in metal coating. In EWF sensing, since interaction with the environment is important, this can only be achieved by thinning the metal coating. Because the EWF has a size of up to several hundred nanometers, the analyte binding leads to extremely small RI change on the surface, resulting in significant changes in optical transmission (Sharma and Mutharasan 2013). The light emission in fiber is explained by wave theory, and the properties of light in the core are determined by the number of modes (N). In multimode fiber, there is a one-to-one relationship between modes and angle of incidence. The number of modes is directly related to a unitless parameter known as the V value as seen in formula I (Buck 1995).

In single-mode fiber with uniform diameter, light is emitted in single mode. However, if the V value changes throughout the fiber due to changes in shape or local RI of the fiber, the single mode fiber may exhibit a multimode fiber property. The matching can be done in modes, in which case the transmission takes place in multiple mode, thereby increasing the evanescence ratio of the transmission. The increase in EWF increases the amount of light that interacts with the surface immobilized molecules, thus achieving the purpose of the analysis for detection.

$$V_{core} = (2\pi/\lambda)\rho[n_{core}^2 - n_{cover}^2]^{1/2} \qquad \text{(I)}$$

(In the formula, $V_{core:}$ The number of modes ρ: The central radius λ: The wavelength of the light n_{core} and n_{cover}: the core and cover of refractive index values, respectively)

Although various methods have been developed to increase EWF in FOBs, the most useful one is conical geometric fibers. There are their some advantages such as low cost, small size, multi-channel and remote sensing (Abdelhaseib et al. 2016).

There are two types of conical fiber optic biosensor (CFOB) geometry useful in today's biosensings. These are tapered ends and continuous tapered fibers (Sharma and Mutharasan 2013). Tapered fibers consist of optical fibers that gradually decrease in diameter to become a micron-size tip. The tapered tip is used as a sensing element. This taper of the tip serves as a conductor for collecting the reactive light, both for receiving light from the light source and for measuring. At the present time, various conical geometry tips such as stepped, conical and combined conical are being investigated extensively for biochemical and clinical applications. The step-shaped tips are produced by dipping into hydrofluoric acid, resulting in a rapid reduction in diameter, resulting in a chemically etched fiber tip. This geometry was found to be of low sensitivity due to the weak V number match leading to a large power loss (Anderson et al. 1993; Kharat et al. 2006). In contrast, the diameter of the elongated tip, while retaining its conical shape, is continuously reduced in the tapered ends and eventually becomes a tip with a nanometer sized radius. Thus, the advanced optical design is obtained. The diameter of the combined tapered ends is reduced non-linearly, which results in a continuous match of the V number, resulting in very low signal loss (Anderson et al. 1993). The biorecognizing molecule is fixed to the conical surface of this region having the smallest diameter, where the strength of EWF is very high. The continuous biconic fibers consist of three regions, which are convergence region or the convex region, the region of fixed diameter called the waist and the region of increasing diameter (Rijal et al. 2005). Because of the waist region in smaller diameter, the EWF is at the maximum level and this region is used for detections. Here, absorption, scattering, fluorescence and resonance changes may occur. Absorption, fluorescence and transmitted light resulting from fluorescence are collected in the region of increasing diameter for the purposes of different diagnostic methods. The two main optical sensing mechanisms of CFOBs are associated with the severity and absorption of light. The sensors based on light severity measurement are ones that have been researched and used today (Sheeba et al. 2005). When the light is passed through a tapered fiber, provided that the analytes attached to the conical surface produce absorption at the wavelength of the transmitted light, the EWF interaction

with the analytes results in a decrease the transmitted light intensity and the magnitude of the decrease is proportional to the analyte concentration. Therefore, the thinning-tapered fibers can be used only by selecting the wavelength of light according to the absorption spectra of the analyte. The magnitude changes in the EWF are determined by measuring the power output changes in a uniform unclad fiber or CFOBs. Factors such as bending, radius, length and taper ratio affect the sensitivity of the CFOBs because the geometric parameters are effective in the performance of density-based sensors. The bending of a tapered fiber increases the EWF ratio in the detection region, thus increasing the measurement accuracy (Díaz-Herrera et al. 2003; Guo and Albin 2003; Mackenzie and Payne 1990; Mignani et al. 1998; Sheeba et al. 2005; Villatoro et al. 2005). Similarly, the smaller radius provides a higher sensibility (Díaz-Herrera et al. 2003). The longer fibers are used to increase the penetration depth at shorter wavelengths (Chen 2004). On the other hand, the tapered region at the bottom also enables the lower row modes to be efficiently matched to the higher row modes in the fiber cover (Guo and Albin 2003).

A light model is to define the relationship of penetration depth with refractive index, conical ratio, length, angle of incidence, numerical aperture and position on conical axis. In this model, the optimal taper ratio (taper diameter/fiber diameter) is required for sensitive response. When the taper ratio is large, EWF can be omitted, but this leads to a decrease in sensor accuracy. Similarly, if the conical ratio is small, the mode propagates so high that it tends to be a free-space wave, causing low signals to be sent to the detector, resulting in a decrease in sensor sensitivity (Ahmad and Hench 2005; Bures and Ghosh 1999). Due to the above mentioned reasons, the choice of the optimum conical ratio takes into account the optical properties of the medium or solution in which the sample is analyzed and the wavelength used.

Currently, FOBs with various antibodies are being developed for the detection of *L. monocytogenes*, other food pathogens, botulinum toxin and staphylococcal enterotoxins. Sample analysis in buffer solutions can be easily performed. If non-target molecules are present in the sample matrix, their non-specific binding to recognition molecules may interfere with detection (Arora et al. 2011).

In a study in which *L. monocytogenes* was determined to be $4.3x10^3$ cfu/ml in medium culture by the combination of FOBs and antibody-based sandwich fluorescence technique, it was found that the sensor was specific to *L. monocytogenes* and is reported that there is no decrease in the received signal strength in the presence of various *Listeria* species and microorganisms in the medium (Aguilar and Fritsch 2003). In addition, in this study, it was remarked that the pathogen was detected by 24 h enrichment from bologna type sausages with hotdog prepared by inoculation of *L. monocytogenes* with another FOB with $10\text{-}10^3$ cfu/g. In another *L. monocytogenes* detection study of ≤10 cfu/ml at count over 20 hours using FOB and EWF, it was reported that enrichment increased antigen expression, an increase in detection occurred, and total detection time after enrichment did not exceed 20-45 minutes (Tims et al. 2001). In a study conducted with FOB developed by using polystyrene guide wave for multiplex determination of *L. monocytogenes* and three other important pathogen bacteria from fast food meat products, it was reported that FOB determined these pathogens multiplex at a concentration of 10^3 cfu/ ml from bovine, chicken and turkey meat samples after inoculated at a level of approximately 10^2 cfu/25 g from each pathogen following to incubated 18 h. Furthermore, it is stated that each pathogen can be identified successfully in less than 24 hours from enriched meat samples with FOB in this study (Ohk and Bhunia 2013). In another study in which the combination of antibody and aptamer was shown to be a functional FOB model, it has been reported that *L. monocytogenes* could be detected as 10^3 cfu/ml in both pure and mixed bacterial cultures with Aptamer-A8, which is a single specific ligand targeted to internalin A in single chain and oligonucleotid structure, and biotinylated polyclonal P66 antibody. In addition, it is stated that this method has the potential to detect the pathogen in case 18 hours of enrichment is made after the inoculation of *L. monocytogenes* as 10^2 cfu/25 g in low rate for ready-to-eat meat products such as sliced meat, chicken and turkey (Ohk et al. 2010).

OBS's superiority in selectivity and sensitivity in the research of foodborne pathogens has put these sensors in the most popular category. Among the OBSs, the FOSs are the first commercial sensors marketed by Research International (Monroe, WA) (Arora et al. 2011). It is reported that *L. monocytogenes* can be detected with a predefined commercial FOS end sensor (RAPTOR™) (Kim et al. 2006). In a hot dog sample with this sensor, it was reported that the pathogen in the buffer solution could be identified as $5.4x10^7$ cfu/ml, and if the flow process was applied, the number could be reduced to $1x10^3$ cfu/ml (Nanduri et al. 2006). In the study of bologna sausages and hotdog ones contaminated at a rate of 10-1000 cfu/g and ones contaminated in a natural way, biotin-streptavidin reaction was used to collect pathogen on optical fiber and the identification was completed by using polystyrene fiber waveguides in less than 24 hours (Geng et al. 2004). In a study in which an optical biosensor was developed to use quantum dots with magnetic nano beads for rapid and specific detection of *L.monocytogenes* and the fluorescence intensity was measured with a probe produced from optical fiber material, *L. monocytogenes* was isolated in very short time as 1.5 hours in decimal dilutions prepared from pure cultures and counted very low concentrations such as 2-3 cfu/ml (Wang et al. 2007).

In conclusion, to date, pathogen determinations have generally been made by performing binding affinity, which can vary or degrade between antibodies and specific antigens. In this sense, biosensors are easy to use, close to real-time results in sensitivity and selectivity comparable to culture methods. With these devices, highly complex samples can be studied without the need for enrichment, without significant reduction in sensitivity or selectivity. Their uses are critical in providing on-site monitoring of pathogens from food matrices. Despite the current research, their potentials can be increased in many respects, especially the specificity of distinguishing target bacteria from multiple organism matrices, direct bacterial detection sensitivity without any enrichment, getting close to real-time results, overcoming natural technical deficiencies, and reducing false positive result numbers. It has become controversial due to the need to check for reliability, robustness and reproducibility in bacterial detection. Another important consideration is the design, which is relatively simple and inexpensive. The conventional enrichment and identification methods of *L. monocytogenes* have proven to be highly sensitive. However, the practical application of these methods is slow due to the multi-step processing of samples during analysis, such as enrichment and separation.

Biosensor-based methods are needed for accurate and rapid detection. Among the biosensors used in identification, optical ones provide better sensitivity than others, especially electrochemical ones, but this method is limited by cost and performance complexity. Their uses are not yet in a specific order and needs to be improved.

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