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Label free Affinity sensing: application to food analysis

Simona Scarano[1], Stefano Mariani[1], Maria Minunni[1,2]

[1]*Dipartimento di Chimica "Ugo Schiff", Via della Lastruccia 3-13, 50019 Sesto F.no (FI), Italy*
[2]*Istituto Nazionale di Biostrutture e Biosistemi, Firenze, Italy*

ABSTRACT
In this paper real-time, label free sensing principle based on Surface Plasmon Resonance (SPR) and gravimetric, i.e. Quartz Crystal Microbalance (QCM) are described and some applications to food analysis problems are reported. Affinity receptors are introduced and examples using antibodies, nucleic acid probes and biomimetic receptors i.e. molecular imprinted polymers (MIP) are reported. In particular pesticides, endotoxins, pathogens, Genetically Modified Organism (GMOs) detection and food origin analysis are reviewed.

Section: REVIEW

Keywords: Affinity-based biosensors; food analysis; Surface Plasmon Resonance; Piezoelectric; GMOs; pathogens

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Corresponding author: Maria Minunni, email: maria.minunni@unifi.it

1. INTRODUCTION

Food and diet are in close relation with human health, and analytical systems aiming to control food quality and safety are always welcome. Food safety is a broader term, which means an assurance that food will not cause harm to the consumers when it is prepared and/or eaten according to its intended use as expressed by the Codex Alimentarius Commission [1]. Food safety is eventually a global issue that affects the health of populations in both industrialized and developing countries [2], becoming one of the highest priorities of public health at national and international level.

Food crises that have occurred over the last 20 years (Bovine Spongiform Encephalopathy, dioxins, foot and mouth disease, etc), changing nutritional habits, new food production processes, increasing international trade and emerging risks, have led consumers to be more sensitive to food safety issues and risk managers to develop and strengthen a more effective food safety system.

At a governmental level, food safety control for public health protection covers the range of different food chains related to a certain food product or product group, including all relevant producers, manufacturing sites and food service establishments within the country as well as those importing into the country. With the increasing International trade in food and the fact that manufacturing sites in one country may provide raw materials to other manufacturers or finished goods (products) for large numbers of consumers living in importing countries, it is critically important that there be a harmonization of food safety control procedures.

The diagnostics industry has focused on the development of analytical methodologies endowed with high sensitivity, speed and portability. In this direction goes the development of fast, user friendly and eventually low cost devices with application to food industry, in field, in supermarkets or, ideally, at consumer places that could be used to obtain information about food freshness, food contamination, food safety, food origin, food content in certain compounds, i.e. allergens, gluten, etc.

In this paper we will introduce Biosensor devices and we will focus on label free and real-time affinity–based sensing. At this regard, some work will be reported using optical and gravimetric sensors for the detection of pesticides, Genetically Modified Organisms (GMOs), and pathogens.

2. BIOSENSORS

Biosensors are devices exploiting biological reactions for detecting target analytes. Such devices couple a biological recognition element (interacting with the target analyte) to a physical transducer, which then translates the biorecognition event into a useful electrical signal [3], [4] (Figure 1). The biological elements immobilized on the sensor can be proteins

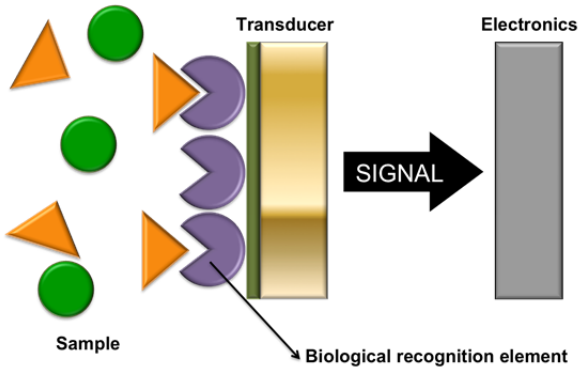

Figure 1. Biosensor scheme: Bio or biomimetic receptors are immobilized on the transduction surface and are responsible of the system selectivity. The binding with the analytes generates an analytical recordable signals whose nature depends on the transduction principle.

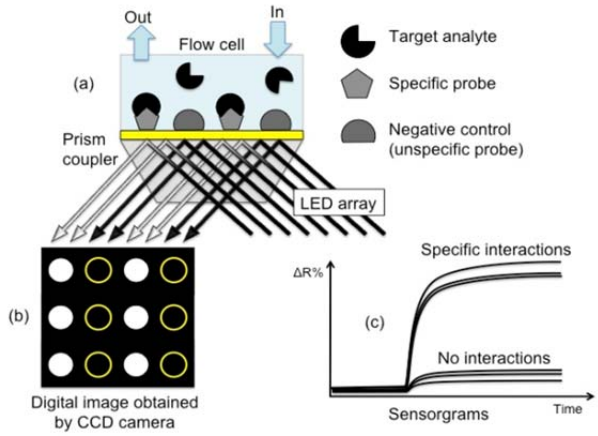

Figure 2. SPRi technology. The receptor is immobilized on the sensor chip gold surface while the ligand is added in solution. From the interaction, a change in the signal is recorded, in label free and real time mode. Parallel analysis on different spots can be performed. The image analysis also visualizes changes on the different spots, providing spatial resolution to the sensorgram changes.

(e.g., an antibody, a cellular receptor), nucleic acids or biomimetic receptors, i.e. aptamers or molecular imprinted polymers (MIPs). The biological element recognizes the ligand by affinity interactions such as immunoreactions, hybridization reactions, or protein/nucleic acids interactions. Depending on the element of transduction, biosensors can be divided into electrochemical, optical, and piezoelectric biosensors, among others. The interaction between some of these biosensors and ligand can be monitored in real time without use of any label, i.e. through label-free detection. Such interactions allow for monitoring the binding event and can be used to evaluate the relative kinetics. For this reason, label free biosensors have been employed for studies of affinity interactions. In particular, with such real-time and label free biosensors, it is possible to evaluate the rate constants for the binding (forward) reaction and dissociation (backward) reaction, represented as k_a and k_d, respectively; and ultimately obtain the affinity constants between the bioreceptor and the ligand expressed as K_A and K_D [5], i.e. association and dissociation constants, respectively.

2.1. SPR-based biosensors

In the field of affinity biosensors, one of the most popular successful transducer belongs to the BIAcore family, which - since early 1990's - revolutionized the way to look at the affinity interactions, by means of coupling optical transduction based on the surface plasmon resonance (SPR) with solid-state chemistry, for the immobilization of biomolecules on the surfaces of gold chip. Over years, the technology of real-time and label-free detection has emerged as a useful and important tool for studying the affinity interactions, between nucleic acids, and nucleic acids and proteins [6]. A further advance in optical label-free sensing has been represented by SPR imaging (SPRi) [7], also known as SPR microscopy [8], first introduced by Rothenhäusler and Knoll in 1988 [9]. Thenceforth SPRi has been applied to investigate interactions of biomolecules arrayed onto the chemically modified metal surfaces. Likewise, SPRi can be made to a real-time, label free method, which additionally allows the simultaneous analysis of many interactions (up to hundreds), by using a CCD camera for signal detection. In such a way, both sensorgrams (i.e., signal vs. time) and SPR images inferred from the arrayed surface can be recorded, which has important impact in high throughput analysis (Figure 2) [10]. Thus SPRi represents an interesting system for multi-analyte detection, allowing parallel analysis of many targets such as different contaminants, i.e. pathogens, pesticides, etc. in food matrices. From a physical point of view, SPR biosensors detect optical signals generated as a consequence of the excitation of surface plasmons (SPs) at the interface of a metal and a dielectric, either a liquid or air. SPs are confined at the interface and they vanish at both sides of the metal surface.

2.2. Piezoelectric-based sensing

Behind optical-based sensing [11], an interesting label free and real-time approach is provided by gravimetric sensors. In literature there is a wide number of papers based on commercially available or in house developed devices, using crystals ranging from 5, up to 10 or 30 MHz, using in QCM in the fundamental frequency or in the relative overtones [12].

Piezoelectric quartz crystals are the basic elements of the quartz crystal microbalance (QCM) device. Quartz resonators are the most used crystals, a crystal variant of Silicon-dioxide (SiO_2).

However, independently from the material uses, the term "piezoelectric" is derived from the Greek word πιεζω ("piezo") meaning "to press". The first investigation on piezoelectricity was performed in 1990 by Jacques and Pierre Curie [13], who observed that a mechanical stress applied to the surfaces of various kinds of crystal caused a corresponding electrical potential across the crystal, whose magnitude was proportional to the applied stress. The Curies also verified the converse piezoelectric effect, in which application of a voltage across these crystals cause a corresponding mechanical strain. This causes a vibrational or oscillatory motion in the crystal, resulting in the generation of acoustic standing waves at a characteristic resonant frequency. The wave is called bulk acoustic wave (BAW) or surface acoustic wave (SAW) in the case of propagation through the substrate or on the surface, respectively. The quartz crystal microbalance is a bulk acoustic wave device based on the converse piezoelectric effect, in which a quartz crystal is sandwiched between two electrodes (Figure 3). The resonant frequency of the quartz crystal depends on several parameters, such as size, density and cut. The most used devices employ AT-cut quartz crystals. AT-cut quartz crystals show a high frequency stability and a temperature coefficient close to zero between 0 and 50 °C [14].

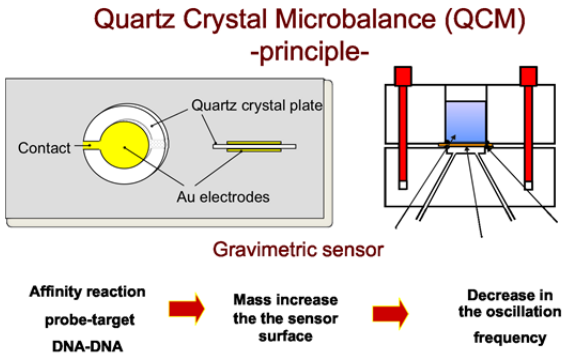

Figure 3. Quartz Crystal Microbalance (QCM): the crystal is sandwiched between two gold electrodes, on which the receptors are immobilized (left). The sensor is inserted in the measuring cell able to operate in static or flow mode (right).

AT-cut crystals oscillate in the thickness shear mode (TSM) [15].

The application of a voltage between the two electrodes causes a shear deformation of the crystal, which is maximized at the crystal faces, making the device sensitive to surface interactions. The resonant condition with the acoustic wave is satisfied by including the crystal into an oscillation circuit, where the frequency of the alternating potential difference applied to the electrodes matches the fundamental frequency of the crystal. The fundamental frequency depends upon the thickness of the wafer, its chemical structure, its shape and its mass [16]. Since the oscillation frequency depends on the crystal mass, deposition of thin films on the crystal surface increases the resonator thickness and decreases the frequency in proportion to the film mass. Measurements of the crystal frequency allow the detection of the film mass; therefore the device operates like a 'microbalance'.

The first quantitative investigation of the piezoelectric effect was performed by Sauerbrey [17], who derived the relationship for the change in frequency ΔF (in Hz) caused by the added mass Δm (in g) in vacuum or in air, as reported in (1):

$$\Delta F = -\frac{2F_0^2}{A\sqrt{\mu_Q \rho_Q}}\Delta_m \,, \qquad (1)$$

where F_0 is the fundamental resonance frequency of unloaded quartz, μ_Q is the shear modulus of AT-cut quartz (2.947×10^{11} g cm^{-1}s^{-2}), ρ_Q is the density of the quartz (2.648 g.cm^{-3}) and A is the surface area in cm^2. The Sauerbrey equation assumes a uniform distribution of mass on the entire electrode portion of an AT-cut quartz crystal. Mass sensitivity decreases monotonically with the radius, in a Gaussian manner becoming negligible at and beyond the electrode boundary [18]. Another assumption of this equation is that the mass added or lost at the crystal surface does not experience any deformation during the oscillation: this is true for thin, rigid layers in vacuum or in air. For thicker, less rigid layers, as it happens for quartz crystals operating in liquid, a more complex theory is necessary. Many factors such as density, viscosity, conductivity and dielectric constant of the liquid may influence the oscillating behaviour. When a quartz crystal oscillates in contact with a liquid, a shear motion on the surface generates motion in the liquid near the interface. The resonance frequency change of a quartz crystal having one face in contact with liquid is described by the Kanazawa and Gordon equation [19], (2):

$$\Delta F = -F_0^{3/2}(\rho_L \eta_L/\pi\,\mu_Q \rho_Q)^{1/2}, \qquad (2)$$

where ρ_L is the density of the liquid and η_L is the viscosity of the liquid. Piezoelectric crystals have been used as microbalance and as a microviscometer owing to their small size, high sensitivity, simplicity of construction and operation, low cost, lightweight and the low power required [20].

3. AFFINITY SENSING

In affinity biosensors, recognition of the analyte in solution by the immobilized biological element is based on an affinity reaction, that could be an antigen–antibody binding, a nucleic acid hybridization, or synthetic receptor/target recognition (molecular imprinted polymers (MIPs), aptamers, or artificial DNA (XNA)). Biosensor selectivity primarily depends on the intrinsic features of the bioreceptor immobilized on the sensing surface. On the basis of these different interactions, the affinity biosensors can be divided into immunosensors, DNA sensors, aptasensors, etc.

An immunosensor uses the affinity interaction between an antigen and an antibody [21]. This interaction has been widely used in food analysis. A recent review reports some interesting application to a wide range of analytes using different transduction principles, both label free and not [22]. Nucleic acid (NA) sensors are based on oligonucleotidic probes and NA hybridization reactions. In NA sensors, artificial nucleosides (XNA) oligomers have also been employed as bioreceptors for application to GMOs detection [23]. XNAs increase sensor stability by avoiding biodegradation or exploiting improved affinity of XNA versus the target sequence compared with conventional DNA probes. D'Agata et al. reviewed works dealing with the great potential of artificial DNA (PNA, LNA, HNA, and MORF) in nucleic acid SPR-based sensing, exhibiting higher selectivity and sensitivity in detecting complementary or mismatching nucleic acid targets [24].

On the other hand, aptamers are single-stranded DNA or RNA (ssDNA or ssRNA) molecules that can bind to pre-determined targets including small molecules, as proteins and peptides, with high affinity and specificity. Such propensity helps code the name of aptamer (from the Latin aptus - fit, and Greek μέρος- part) [25]. These nucleic acid aptamers have been engineered through repeated rounds of *in vitro* selection or equivalently, SELEX (Systematic Evolution of Ligands by Exponential Enrichment). This class of binders plays a role in bioanalysis [26]−[28] as receptors in heterogeneous phase-based assays [29], [30], in biosensors, in affinity chromatography, in Enzyme Linked Aptamer Assay (ELAA), in macroarrays [31], [32], as well as in solution as molecular beacons [33].

Aptamers are obtained through a combinatorial method alternating steps of selection and amplification. Initially, a combinatorial library of oligonucleotides (most typically 30 to 50 bases) is constructed and improved variants are identified through an iterative selection process performed in vitro, the SELEX process, which involves iterative cycles of selection and amplification (usually 12-18 cycles). During these cycles, the nucleic acid molecules with the highest affinity for the target are separated from the nonbinding species. The enriched library (10–30 individual sequences) is finally cloned and sequenced, and the single sequence with the highest affinity for the target molecule is isolated.

Aptamers exhibit strong affinity and high specificity for a predetermined target.

Behind NA aptamers, artificial combinatorial proteins, consisting of a variable peptide sequence inserted within a

constant scaffold protein, have been reported. In analogy to the nucleic acid counterpart, they are called Peptide Aptamers [34], [35].

4. ASSAY DESIGN

Similar to other bioanaytical based assays, affinity sensing is generally a performer using two assay formats, direct and indirect, were mostly adepted on SPR or QCM biosensor for the detection of analytes in food analysis (Figure 4).

The assay selection is based on the molecular weight (MW) of the analyte, on the bioreceptors/analyte affinity, and on the matrices under investigation. In the direct assay, bioreceptors are immobilized on the sensing surface and analytes are added in solution. This strategy is usually adopted for analyte with high MW, sufficient to develop a refractive index variation and resulting in detectable signal. Indirect assays are preferred for low MW analytes where purified analytes (or conjugates) are immobilized on the sensor surface, while specific bioreceptors are incubated with the samples to be analyzed and injected to the sensing surface; when the solution reaches the surface, only the remaining free bioreceptors bind to immobilized analyte, providing a signal inversely proportional to its concentration in the sample. In both cases, i.e. direct and sandwich-based detection, secondary bioreceptors are exploited in multi-step assays to improve detection limits [21].

4.1. Pesticides

Pesticides are relevant pollutants due to their large amounts released into the environment. For this reason, many countries have established maximum residue levels (MRLs) in food products [36]. Drinking water analysis can be achieved by SPR sensing. The first example for sensitive, selective analysis of atrazine was early reported by our group where an indirect immunosensor was developed thanks to the availability of monoclonal antibodies (Mabs) developed in mice; ppb levels of detection were achieved [37] with very good selectivity tested with terbutylazine and simazine. Later other SPR immunosensor examples were eventually reported detecting atrazine with a detection limit of 20 ng/L (ppt) for the optimized assay, and results validated by gas chromatography coupled to mass spectrometric detection (SPE-GC-MS). Another SPR affinity sensor, still for atrazine, was realized by a direct detection system for herbicides, inhibiting photosynthetic electron transfer developed using the photosynthetic reaction

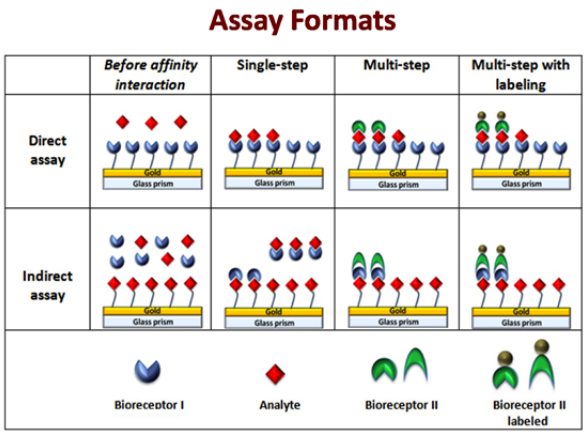

Figure 4. Schematic representation of different strategies for biomolecular recognition, i.e. by direct and indirect detection. Both approaches can be based on single- or multi-step assays, with or without the presence of labels.

centre (RC) from the purple bacterium, *Rhodobacter sphaeroides*, immobilized on the chip and atrazine in solution [38]. Atrazine was detected down to 1 μg/ml and, to evaluate the binding specificity to atrazine, chlorinated aromatic herbicides, (DCMU), N'(3,4-dichlorophenyl)-N,N-dimethylurea and 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP) were investigated as well as other water pollutants, i.e. dioxins, polychlorinated biphenyls (PCB) [39]–[41]. Similarly, competitive immunosensing for parallel analysis of endocrine-disrupting compounds (atrazine, 2,4-dichlorophenoxyaceic acid (2,4-D), 4-nonylphenol, and benzo[a]pyrene) to respective protein-analyte conjugates was realized [42], [43]. An analysis time of 45 min (including 30-min incubation of the sample with antibodies) and limits of detection as low as 0.05, 0.07, 0.16 and 0.26 ng mL^{-1} (ppb) were demonstrated for benzo[a]pyrene, atrazine, 2,4-D and 4-nonylphenol, respectively [43]. 2,4-D immunosenensing has been eventually set up with several mAb clones set up using QCM transduction.

Biomimetic receptors were used as recognition elements, instead, when the dendritic nano-fibers based piezoelectric sensor was developed for the analysis of dichlorodiphenyltrichloroethane and hexachorobenzene prevalent together as organochlorinated pesticide residues in real samples (human blood serum, and river water samples) [44]. Detection limits of dichlorodiphenyltrichloroethane and hexachlorobenzene were realized as low as 0.75 and 0.69 ng mL^{-1} (ppb) and linearity observed in the concentration ranges 5.0–150.0 and 5.0–75.0 ng mL^{-1}, respectively.

4.2. Endotoxins

Endotoxins, chemically lipopolysaccharides usually found in food, environment, and clinical products of bacterial origin, are unavoidable ubiquitous microbiological contaminants [45]. An interesting review describes the application of different sensing principles including SPR and QCM for the detection of endotoxins. Detection of endotoxin is essential for quality control in biological products, medical devices, serological products, parenteral drugs, recombinant therapeutic products and food and water security. *Escherichia coli* and *Salmonella paratyphi* produce endotoxins causing sepsis, cholangitis, and neonatal meningitis. The contact with population may come from contaminated food and water.

Reports of reusable QCM based sensors for detection of staphylococcal enterotoxin A have been established [46]. Endotoxin detection using QCM with a detection limit of 0.01 pg/ml has been accomplished [45]. Most of the mentioned application deals with the use of Antigen-Antibody binding, i.e. immunosensing. However, as mentioned before, nucleic acid probes can also act as bioreceptors in affinity sensing targeting DNA or RNA sequences as analytes of interest.

From late '90 we have assisted to the increase of nucleic acid based sensors where a molecular probe is immobilized on the surface and the interaction with the complementary analyte leads to the hybridization reaction with resulting double helix formation.

4.3. Genetically Modified Organisms (GMOs)

An interesting problem studied by our group is the detection of genetically modified organisms (GMOs).

With the advent of biotechnology, new "products" have appeared such as GMOs. This achievement has been realized by manipulating the DNA molecule, by introducing new genes into a host, which would never naturally exchange the DNA

with the donor. GMOs are defined as living organisms whose genome has been altered in a way that does not occur naturally by mating or natural recombination. The modification involves the introduction of an exogenous sequence in the host genome - either capable of expressing an additional protein that confers new characteristics, or able to hybridize specifically to target m-RNA, inactivating it (e.g GMOs with delayed ripening). The foreign DNA is "usually" inserted in a gene cassette consisting in an expression promoter (P), a structural gene ("encoding region") and an expression terminator (T). One or more cassettes can be introduced into the host genome [47], Figure 5.

Analytical approaches based on DNA detection of GMOs are based on the detection of markers of transgenosis, i.e. one of afore mentioned elements, the promoter, the terminator or the coding gene [48]. The gene cassette (one or more, i.e. cassettes) is built up by assembling some DNA sequences, with a known function, coming from different species. So a cassette can be made by assembling bacterial or viral DNA (generally for the promoter and terminator region) together with an eukaryotic DNA codifying for the inserted gene conferring the desired characteristics. In patented GMOs very established and stable promoters and terminators cassettes are generally cloned. The promoter of the subunit 35S of ribosomal RNA of the cauliflower mosaic virus (P35S) and the terminator NOS from *Agrobacterium tumefaciens* are widely used for the production of many transgenic vegetables, such as soy Roundup Ready™, maize MaisGard, and the tomato Flavr Savr.

This has generated an interesting debate on the use, application, risk and analysis of GMOs. For this, there is an increasing number of papers dealing with GMOs detection, since many countries have strict legislation worldwide allowing only the presence of GMOs within a certain threshold, and requiring labelling of transgenic organisms at all stages of their placing on the market. Reliable and sensitive methods for GMOs detection, from protein to DNA based methods have been reported in the last decade. In particular, several biosensors were developed, using different transduction principles, over the last fifteen years, targeting the genetic sequences identified as markers of transgenosis, i.e. the promoter and the terminators ones. This work has been very nicely summarized in a recent review [49]. In particular SPR [50], [51] and QCM-based sensing [52] have been developed by our group targeting both P35S and TNOS sequences previously

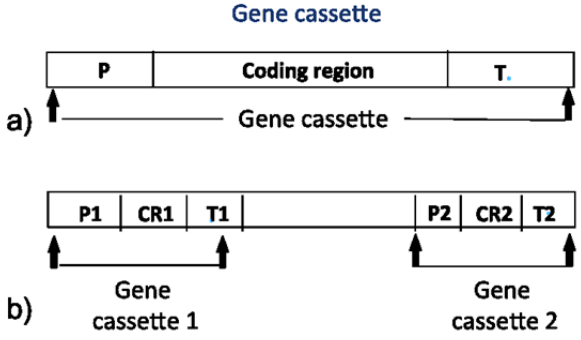

Figure 5. The insertion of exogenous genes into the host genome comprises an element called promoter (P) allowing the expression of the coding region (CR) containing the modification i.e. gene or sequence, with desired characteristics to be introduced into GMOs. Finally a terminator (T) is added. These three elements P, CR , T are defined "gene cassette". In GMOs one or more gene cassettes can be present.

extracted, fragmented, and denatured [53], [54]. Specific sequence detection has been achieved in PCR amplified samples and in genomic DNA (10 ppm) by using as negative control the wild type genome (i.e. not genetically modified) of *Nicotiana glauca*. The European Union (EU) has elaborated a legislation for genetically modified (GM) food control, which establishes both the legal basis for the approval procedure of GMOs and the post market traceability and labelling requirements for GMOs and GMO-derived food and feeds [55], [56]. In this perspective, the application of biosensors to real matrices analysis for GMOs detection has been successfully attempted, as well as the monitoring of the GM soya food processing (from seed to end product) in a pilot plan where the presence of target sequences in soya was followed over steps [57].

It should be noted that correct traceability of the transgenic events in food processing can be achieved only by monitoring target sequences during the whole manufacturing process, where the target analytes are followed from the initial source to the end product, in a systematic and controlled manner passing through a series of intermediate phases. In fact, false negatives can arise in processed food. Several factors can negatively affect the detection of transgenes, on dependence of the considered matrices (mechanical procedures i.e. milling, thermal treatments, chemical degradation, presence of inhibitors of the PCR reaction, etc.). Moreover, the use of harsh conditions in the processing steps could impact on the integrity of genomic DNA, which represents the starting point of the whole analysis [58]. It is thus of primary importance to verify the possibility to amplify (amplificability) the extracted DNA. The DNA degradation can be caused, for example, by heating treatments [59] and exposure to pH changes [60]. Furthermore different inhibitors of the Taq polymerase enzyme such as proteins, fats, polysaccharides, polyphenols, and other compounds that may be present in DNA may inhibit the DNA template amplification [61]-[64]. To manage these problems, the traceability of several selected DNA fragments differing in length, mapping on the inserted cassette, was studied along a complete industrial soybean processing chain (Figure 6). In particular, samples were collected from the different levels of an industrial soybean processing plant working with 80 % GM RoundupReady-soybean. The genetic modification is the Roundup Ready-soybean event GTS 40-3-2 (notification C/UK/94/M3/1 of Monsanto). Eventually, DNA extraction procedures from the different matrices and PCR amplification of selected fragments differing in length (Figure 7) were set up.

As expected, the amplificability of the fragments was inversely related to their size since the degree of template DNA integrity affected the success of the PCR reaction, especially in the case of long amplicons. The QCM affinity sensor, carrying a P35S immobilized probe on the sensor chip, was able to detect the 195 bp fragment (P35S) complementary sequence in the samples selected along the complete industrial soybean processing chain. This sequence was confirmed to be present amplified in the samples by end-point PCR analysed in parallel by gel electrophoresis.

More recently, other authors reported about optical based sensing for ultrasensitive genomic DNA detection of transgenes sequences. In particular SPR imaging (SPRi) [65], the evolution of conventional SPR instrumentation, with the advantage of parallel analysis of many targets, has been applied, or by optic fiber confirming the possibility of detecting directly, by passing

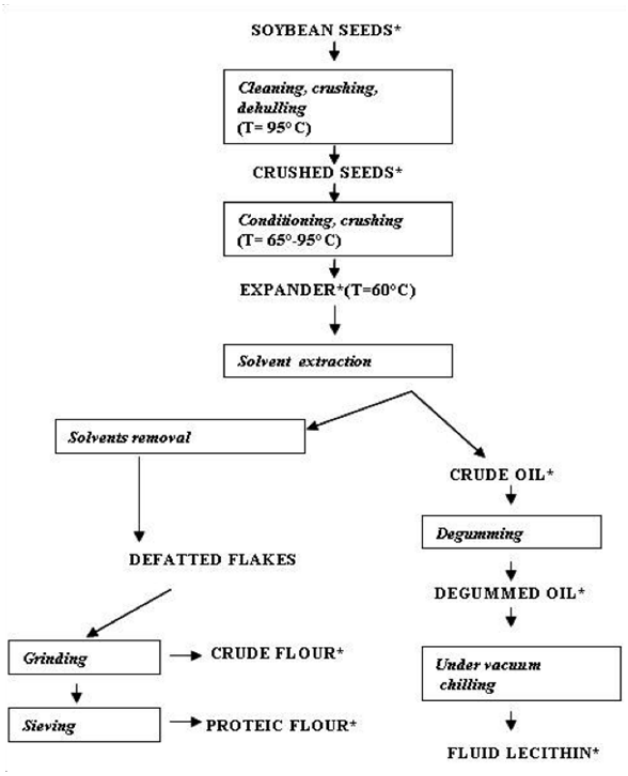

Figure 6. Sampling plan along the complete industrial soybean processing chain. Samples were collected at Pilot industrial plan with the help of Dr. R. Onori, Istituto Superiore di Sanità- ISS, Rome, Italy.

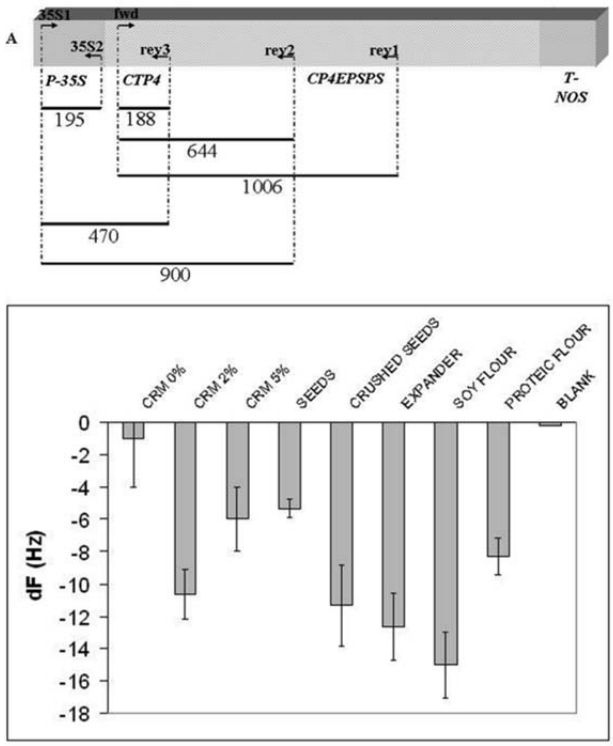

Figure 7. Fragments selected for the traceability of the transgene along the industrial soybean processing chain, spanning from 118 bp to 1006 bp in length, allowing to evaluate the optimal length to be traced in the different resulting matrices (upper). QCM-base sensing detection of P35S sequence (195 bp) in the samples collected along a complete industrial soybean processing (lower).

the amplification step, GMOs using label free, real time nucleic acid-based sensing [23].

4.4. Detection of food origin

Species-specific sequences can be targeted for the identification of food origin. Modern methods for meat identification are based on DNA analysis. This is the case of a study conducted on highly repeated sequence called satellite 13, present in *Bos taurus* [66]. In the bovine genome, eight highly repetitive and several minor repetitive sequences have been detected comprising a total of 27 % of the DNA [67]. They allow species-specific DNA sequence identification, which has some advantages over protein analysis [68]. Molecular biology methods allow the determination of DNA also in heat-treated nourishment and are, therefore, suitable for the identification of species-specific DNA in meat and bone meal and concentrate mixture [69]. DNA analysis discriminates between related species, such as sheep and goat or chicken and turkey [70]. By QCM based–sensing it is possible to detect highly repetitive and species-specific DNA sequences present in bovine (*B. taurus*) genomic (non-amplified 10 μg/ml DNA) DNA, extracted from meat samples. The selectivity of the sensor was tested with porcine (*Sus scrofa)* DNA, and no interaction was observed.

Similar results were obtained by SPR sensing using the same microsatellite sequences [54].

The system is able to discriminate between bovine and pork meats in real samples. This approach can be transferred to any other sequence since the system selectivity relies on the probe immobilized on the sensor surface.

4.5. Microbial contamination

Ideally, the success of food safety management should be reflected in the health status of the population concerned.

Epidemiological data show that major outbreaks of food safety problems persist, e.g., the contamination of ground turkey by *Salmonella enterica* serotype Heidelberg in the USA (US Centers for Disease Control and Prevention, 2011) and the contamination of sprouts by *Escherichia coli* O104 in Europe [2]. Moreover, the most recent data on zoonoses, collected in the European Union in 2011, showed that confirmed cases of human campylobacteriosis were reported and this number has followed a significant increasing trend in the last 4 years. Moreover, Verotoxigenic *Escherichia coli* human cases has been increasing in the EU since 2008. The number of salmonellosis cases in humans decreased in 2011 by 5.4 % compared with 2010, but still 95,548 confirmed human cases were reported.

Interesting work has very recently been reported using Molecular imprinted polymers for *E.coli* detection by QCM and SPR sensing. Amino acid based recognition element, N-methacryloyl-L-histidine methylester (a polymerizable form of histidine) was used for MIP synthesis [71]. Response times (time required to reach 95 % of steady state) were calculated as 113 s for SPR and 56 s for QCM so both systems are very rapid compared to the classical bacterial culture methods which may take up to 7–8 days for the answer. The limit of detection (LOD) and the limit of quantification (LOQ) of the QCM system were found as 3.72×10^5 CFU/mL and 1.24×10^6 CFU/mL, respectively. LOD and LOQ of SPR system were also calculated and were found as 1.54×10^6 CFU/mL and 5.13×10^6 CFU/mL, respectively. Spiked apple juices samples were reported. LODs for bacterial detection *E. coli*, *Salmonella thyphimurium*, span from 10^2 CFU/mL to 10^8 CFU/mL as indicated by these authors. Affinity interactions are also involved in the case of mannose-containing oligosaccharides with the fimbrial lectin of *E. coli* and this property can be used for designing novel biosensors. Modified carbohydrate ligands

were synthesized and immobilized onto gold electrodes and SPR surfaces. A detection limit of 1 CFU/mL was reported for this bacterium. The relative selectivity of these ligands for *E. coli*, *Citrobacter freundii*, were 100 %, 2.6 %, and 8.6 % respectively. The biosensor was validated using spinach leaves at 3.0 CFU/mL [72].

Immunosensing has been reported using SPR for *E.coli* O157 H7, *Salmonella Enteritis* and *Lysteria monocytogens* detected in culture media [73]. LODs were 0.6×10^6, 1.8×10^6, and 0.7×10^7 CFU/mL, respectively, in the presence of non-target pathogens at concentrations of 10^5 to 10^8 CFU/mL. Displacement assay applied to QCM for *Listeria* was also reported with a working range between 10^5–10^7 and applied in milk (2 % fat) [74]. Using nucleic acid QCM sensing, bacterial detection can be also achieved as demonstrated for *Staphylococcus aureus* (MRSA) and antibiotic resistance gene can be eventually targeted i.e. gene mecA, codifying for an anomalous protein [75].

SPR nucleic acid sensing using a sandwich format for invA gene of *Salmonella thyphimurium*, using both DNA and aptamer probe with streptavidin (SA) as mass enhancer, has been very recently reported [76]. This strategy was successfully applied to the determination of *Salmonella* at levels as low as 60 CFU/mL. Comparing to *Salmonella*, no significant response was obtained when *E.coli*, *S. aureus* and *S. pneumoniae* were tested using the same assay. Furthermore, the sensor chip could be regenerated 200 times.

5. CONCLUSIONS

Affinity sensing can be achieved using different bioreceptors immobilized on the chip. Most frequently used are antibodies (or antigens) and thus immunosensors are developed. Since early '90 nucleic acid sensing has appeared as an interesting approach for target sequence detection, and more recently other biomimetic/synthetic receptors (aptamers, MIP) are used to account for harsh conditions where bioreceptos may be not the optimal choice. Here we reported about two popular detection principles in label free and real time sensing which can be indifferently used as demonstrated in provided examples. This paper is far from exhaustive since vast literature is available, but the aim was to provide some applications for different classes of analytes of interest in food analysis, starting from the direct experience with both techniques coupled to immuno and nucleic acid sensing.

In conclusion, affinity biosensors are flexible devices that can be adapted to the analytical problem, which can be addressed by selecting the suitable receptor to achieve the desired sensitivities and selectivities. They can work in real matrices and can be coupled to on line monitoring or for spot measurements. They can be portable or miniaturized. These characteristics make them interesting devices in food analysis.

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