**Application of Biosensors in Milk and Some Selected Milk Products Packaging: A Review**

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**Abstract**

Milk and milk products are excellent sources of casein, fat, lactose, vitamins (B2 & B12) and minerals (Ca & P). These can be contaminated naturally or intentionally by different additives and adulterants occurring during various processes during handling, processing, packaging, storage, transportation, distribution and sale of milk and milk products. It is important to detect the harmful contaminants to ensure the consumers’ safety and health. Among various sophisticated and sensitive instruments, biosensor seems to be an effective analytical tool to detect and measure milk contaminants in package environment. In the present review, different types of biosensor on the basis of Bio-receptor and Sensor Elements of Transducer have been described which can be used for the detection of contaminants. Moreover, the available literature related to the detection and measurements of contaminants by using different types of biosensor technology contained in milk and milk products packaging such as antibiotics, drugs, pesticides, heavy metals, toxins, pathogens, biomolecules, allergen and additives have been reviewed in the present investigation.

**Keywords:** Biosensor, Milk and Milk Product Packaging, Milk Contaminant, Analyte, Bio-receptor

**1. Introduction**

Nutritional quality attributes and consumer acceptability of milk and milk products is characterized by appearance, texture, odor and taste. Milk analysis is an important aspect to protect the consumers ‘health against any adulteration, spoilage or contamination. It also helps to detect natural components such as sugars, amino acids, alcohols and additives (e.g. vitamins and minerals). Milk and its products may be contaminated or vitiated at various stages of production, handling, processing, packaging, storage, transportation and distribution. It may be contaminated by unacceptable quantity of harmful additives and adulterants such as toxins, pesticides, antibiotics, heavy metals and drugs during poor agricultural, veterinary and industrial practices. Pathogenic bacteria and viruses can also contaminate and spoil the milk and thereby making it unhealthy for human consumption (Fischer et al 2011).The major contaminants and their maximum residual limits have been presented in Table 1 (Khaniki 2007). Therefore, detection and control of chemical and biological hazardous contaminants of milk and milk products by selective, sensitive and reliable analytical methods is urgently required for safety of consumers’ health. Nowadays, many innovative technologies and sophisticated instruments are being used for milk analysis such as liquid chromatography, gas chromatography, atomic absorption and mass spectrometry. All these technology are very expensive, not portable and require an experienced technician. Therefore, application of biosensors seems to be accurate and promising screening tool because it is a highly sensitive and highly specific tool with fast readout times and simple sample pretreatment. It requires low cost for mass production (Antonacci et al 2016). There are several companies available worldwide who are involving in manufacturing of different types of biosensor (Figure 1). The present article has been reviewed for the application of biosensors in detection of various chemical and biological contaminants in packaged milk and milk products. In many public and private sectors in dairy industries, the focus has always been towards the selection of simple, sensitive, rapid, reliable and cost-effective biosensors to monitor health hazardous organic and inorganic chemical contaminants in dairy products.

**Table 1:** Major milk contaminants and their maximum residual limit (Khaniki 2007)

|  |  |
| --- | --- |
| **Milk contaminant** | **Maximum Residual Limit** |
| Antibiotic (µg/kg) | Benzyl penicillin | 4 |
|  | Tetracycline | 100-200 |
|  | Oxytetracycline | 100 |
|  | Chlortetracycline | 100 |
|  | Trimethoprim | 50 |
|  | Ceftiofur | 100 |
|  | Streptomycin | 200-1000 |
|  | Oxfendazole | 10 |
|  | Sulphonamide | 100 |
|  | gentamicin | 200 |
|  | Erythromycin A | 40 |
|  | Spiramycin | 200 |
|  | Colistin | 50 |
|  | Kanamycin A | 150 |
|  | Neomycin B | 1500 |
| Insecticide (mg/L) | Cypfluthrin | 40 |
|  | Cypermethrin | 50 |
| Antihelminthic agent (mg/L) | Thiobendazole | 100 |
|  | Albendazole | 100 |
| Pesticides (mg/kg of milk fat) | Hexachlorobenzene | 0.8-7 ng/g |
|  | Aldrin-dieldrin | 0.0074-0.0271 |
|  | Hexachlorocyclohexane | 0.094 |
|  | DDT | 0.159 |

**2. Biosensor and Biosensor-Packaging System**

Biosensor is a combination of biotechnology and electronics. It is an analytical device which converts the response of bio-receptor test analytes interaction into a measurable signal which is directly proportional to the concentration of the analytes to be tested. In a simple way, biosensor is a technique which converts biological signals into electrical signals such as current or voltage by physicochemical detector (Anjum and Pundir 2007). Professor Leland C. Clarke Jr. is known as father of biosensor because of his innovation of oxygen electrode “Clarke electrode” in the year 1956 for detecting oxygen in water, blood or other fluid samples. Clark and Lyons developed the first biosensors (Clarke and Lyons 1962).Immobilization and transducer are two critical components of biosensors in converting biological response of test analytes and biological recognition element interaction into measurable signals. The interaction and further recognition is basically based on the affinity between antigen and antibody reaction, enzyme substrate reaction, receptor hormone reaction etc. Immobilization of biological recognition element can be performed by various procedures such as entrapment, adsorption, encapsulation, covalent bonding and cross linking. These are conventional practices which create problems in long term stability and therefore in the recent period, with the advancement of nanotechnology; nano-materials are being used to achieve improved immobilization for reliable, stable and selective biological recognition element such as nanoparticles, nanotubes, nanorods and fiber optics Moreover,a lot of biological active compounds such as antibodies, enzyme, nucleic acids, lectins, tissues, cell organelles and microorganism are immobilized for biosensing. A transducer is a physico-chemical detector and current potential absorption of light which detects records and transmits the qualitative and quantitative information of physicochemical process occurring between test analyte and bioreceptor (Clarke and Lyons 1962). Several research works are being carried out in developing biosensors for selective detection of wide range of contaminants based on the types of bio-receptors and sensor elements of transducers in order to achieve the ideal characteristics of biosensor such as it should possess high linearity to detect high substrate concentration, it should be sensitive and selective so that interference by chemicals used is minimized to get accurate result and it should also have 95% response time The components and detailed work flow of a typical biosensor has been presented in the Figure 2.



**Figure 2.**The components and detailed work flow of a typical biosensor

The biosensor-packaging system monitors and provides information about internal environment of package and status of product packed to the external databases. In this system, intelligent packaging is used with biosensor and data carrier elements to monitor the internal package environment such as temperature, time, pH, and moisture content. Biosensor is used on labels or package as smart chips or micro/nano-sensitive elements.

Three types of biosensor-packaging system have been reported (Takhistov 2009):

1. Off-package system- In this system, biosensor is not directly attached with packaging material or package content.
2. On-package system- In this system, biosensor is attached with packaging material as discrete or distributed form. RFID is an example of on-package system.
3. In-package system- In this system, biosensor is kept inside package as discrete or distributed form.

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**3. Types of Biosensor Based on Bio-receptor**

**3.1 Microbial Biosensor**

Microbial biosensor is one of the most useful devices to monitor environmental, food, fermentation and clinical samples. It is an analytical device used to immobilize microorganisms onto a transducer for the detection of target analytes (enzymes of microorganism acts as Bio elements). In the microbial biosensor enzymes of living microbial cells act as biological recognition (Shahbazi et al 2016). Microbial biosensor may be of three kinds such as fluorescent microbial biosensor, colorimetric microbial biosensor and bioluminescent microbial biosensor. In all the three types, optical transducers use optical properties i.e. fluorescence, adsorption and bioluminescence to produce measurable signals according to the concentration of bioelements. In the fluorescent microbial biosensor, fluorescence is produced by the expression of fluorescent protein which is encoded by reporter gene (green fluorescent gene, *gfp*) fused with inducible promoter in the genetically modified microbial genome such as *E. coli* fixed on optical transducer. In the colorimetric microbial biosensor, microorganisms hydrolyze specific compounds in to chromophoric product which is measured by colorimeter. For the detection of methyl parathion, Kumar et al (2006) and Kumar and D’Souza (2010) have utilized *Flavobacterium* sp. and *Sphingomonas* sp. respectively on optical transducer which has hydrolyzed the substrate in to a chromophoric para-nitrophenol to be measured by colorimeter.Bioluminescent microbial biosensor uses lux gene of living cell which encodes for luciferase enzyme responsible for bioluminescence generation.

**3.2 Nucleic Acids Based (NAB) Biosensor or Genosensor**

This type of biosensor utilizes oligonucleotide probes of DNA (deoxyribonucleic acid), RNA (ribonucucleic acid), PNA (peptide nucleic acid) and aptamers which are more akin to antigen-antibody or receptor ligand binding remained immobilized on a solid support and coupled with transducer. DNA is widely used oligonucleotide probes among other NAB probes that follow the base sequence complementarity for signal generation. Fundamental principle behind DNA based biosensor depends upon the sequence complete chargaff rule except aptameters. DNA is consisted of a double stranded sugar phosphate backbone; RNA has a single stranded sugar phosphate backbone and PNA having repeating N-(2-aminoethyl)-glycine units linked by peptide bonds. Aptamers are short and single-stranded (40-60 nucleotide long) strand of DNA and RNA both which is obtained synthetically by systematic evolution of ligands by exponential enrichment process (SELEX).

**3.3 Antibodies Based Biosensor or Immune-sensor**

An immune-sensor is a biosensor where transducer creates electrical signal by detecting the formation of antigen- antibody complexes on platform. This biosensor measures a wide range of virulent pathogens (bacteria, fungi, viral, parasites) and their toxins such as mycotoxin, marine toxin etc. in order to prevent hospital acquired and fatal infections and assures general public health and safety by utilizing polyclonal, monoclonal and recombinant antibodies on electrical, piezoelectric, optical, magnetic and thermal platform. Antibodies are ideal recognition elements that provide sensors with high specificity and sensitivity. Immuno-sensors also used for diagnostics for detection of plethora of analytes disease markers.

**3.4 Enzyme Based Biosensor**

Enzyme based biosensors are essential in the development of drugs. Enzyme based biosensor responds faster due to shorter diffusion path. The pharmacological agents are activators and inhibitors of enzymes.

This type of biosensor is designed using enzymes as bioreceptor such as glucose oxidize polyphenol oxidase and urease which is specific to substrate and converts this into products. In the enzyme based biosensor, an enzyme is placed very closely to an electrode surface. This enzyme then catalyses the reaction by consuming electro active reactant and thus generates electro active product. The depletion of reactant or production of products is then monitored and measured. The quantitative measurement indicates the equal proportion of analyte concentration. The identification of target molecules is performed by following ways

* Analyte detects enzyme activation/inhibition.
* Sensor detects products produced by enzyme catalyzed reaction.
* Sensor detects modification in enzyme properties after reaction.

Signal amplifying has been improved by combining enzymatic reaction with redox cycling. This type of biosensors is highly selective, sensitive, and rapid and identifies a wide range of chemical targets such as substrate, products, inhibitor, and modulator. In the enzyme catalyzed chemical reaction, enzyme is not involved therefore in this type of biosensor, enzyme could be used unlimited times to monitor target analytes. But due to limited stability of enzymes this type of biosensors has limited life span. The disadvantage with these biosensors is that it is very expensive due to isolation process of enzymes and enzymatic catalytic activity could be deactivated due to non-appropriate storage and during process of immobilization (Rathee et al 2016).

With the recent advances in enzyme based biosensors, certain enzymes are being used which emit photons as by-products of the reactions. By this way biosensor detects the biological active elements by the process of bioluminescence by using an instrument called luminometer. In the living cell, ATP can be detected by using Luciferase-luciferin complex (enzyme-coenzyme conjugate). This enzyme emits light and is found in *Photinuspyralis*, a fire fly. Total light output is directly proportional to the ATP of the sample. This biosensor requires 104 cells to produce detectable signal. Similarly in bacterial cell, *lux* gene emits lights. This gene has been cloned first with phage nucleic acid then during bacterial infection this gene has been incorporated with bacterial genome. Upon expression, the emitted light has been detected by luminometer. The detection limit of this type of biosensor is 1x102 cells/60 min (Mandal et al 2011).

**3.5 Cells and Organelles Based Biosensor**

This biosensor utilizes the integration of living cell or its organelles such as mitochondria, endoplasmic reticulum, Golgi body, nucleus, nucleolus etc. on the biosensor platform for detection of multiple analytes from clinical, environment, food, milk and other sources. The whole cell includes prokaryotes (such as bacteria) and eukaryotes like vertebrates, invertebrates and yeast.

**3.6 Protein and Peptide Biosensors**

This biosensor utilizes proteins and peptides as bio-recognition elements to detect various test analytes such as DNA, RNA, proteins, enzymes, bacteria, metal ions and antibodies.

**4. Types of Biosensors Based on the Sensor Elements of Transducer**

**4.1Piezoelectric Biosensor or Acoustic Biosensor (Sound Vibration based Biosensor)**

Piezoelectric biosensor measures the electric signal produced by the piezoelectric substance such as quartz crystal, when a mechanical force is applied under the influence of electric field. This force changes the mass of crystal structure and that creates the oscillation frequency to be recorded by biosensor. The change in oscillation frequency is directly proportional to the mass of sample analyte on crystal structure. For the detection purpose, a biocapture material such as antibody (Ab) is applied on crystal surface. This biocapture material possesses specific binding with the sample analyte such as antigen (Ag). This specific Ag-Ab interaction on crystal structure creates oscillation frequency to be recorded by detector. The principle of oscillations change is due to mass bound on the piezoelectric crystal surface. In this biosensor, gold detects the specific angle for emission of electron waves which is emitted from the substances exposed by quartz crystals and laser light. This biosensor is of two types first is quartz crystal microbalance (QCM) and second is surface acoustic wave device. QCM measures the change in [frequency](https://en.wikipedia.org/wiki/Frequency) of a [quartz crystal](https://en.wikipedia.org/wiki/Quartz_crystal) resonator to investigate interactions between bio molecules. It is useful for monitoring the rate of deposition in [thin film deposition](https://en.wikipedia.org/wiki/Thin-film_deposition) systems under vacuum and the [affinity](https://en.wikipedia.org/wiki/Chemical_affinity) of molecules (eg. Protein, Virus etc) to surfaces functionalized with recognition sites in liquid. However, a surface acoustic wave (SAW) has a [longitudinal](https://en.wikipedia.org/wiki/Longitudinal_wave) and a vertical shear component. SAW when couples with any media in contact with the surface affects the amplitude and velocity of wave, and this way SAW sensors directly sense mass and mechanical properties (Vo-Dinh et al 2000).



**4.2 Magnetic Biosensor**

This biosensor utilizes paramagnetic or super paramagnetic particles from nanometres’ to microns sized to measure changes in magnetic properties or magnetically induced changes. For detection purpose, magnetic particles and surface of sensor are coated with bio receptors such as DNA, RNA, antibody etc. The bio receptors bind specifically with test analyte. The coated magnetic particles move towards test analytes by influence of magnetic fields and after a definite time period coated magnetic particles with attached test analytes bind to the surface of sensor and unbound/ unattached magnetic particles with test analytes are removed from the surface. The number of bound magnetic particles with analytes gives the measurement of the concentration of test analyte. The magnetic markers can be manipulated inside micro fluidic channels by high gradient magnetic fields that can in turn be detected by magnetic sensors (Chon & Li 2008).

**4.3 Optical Biosensors**

Optical biosensors are made up of a light source, optical components for specified light beam generation, a modulating agent, a modified sensing head and a photo-detector. This biosensor measures the changes in light intensity. It determines changes in light absorption between the reactants and products of a reaction of a reaction, or measures the light output by a luminescent process. The most common use of this technology is for whole blood monitoring in diabetes control (Domborsky et al 2016).

**4.4. Fluorescent Biosensors**

Fluorescent biosensors consist of fluorescent probes which are mounted genetically, enzymatically or chemically with bio-receptors. Probes may be cell metabolite, biomarkers and ions. Bio-receptors identify test analytes such as serum, cell extract and transducer generates readily detectable fluorescent signal of status or activity of target analytes, gene expression, transcription, translation, protein localization, signal transduction, cell cycle etc. Fluorescent biosensors also allow researchers to quantify protein activity (Van Engelenburg & Palmer 2008).

**4.5 Potentiometric Biosensor**

 This biosensor measures the change in electric potential between ion/gas selective electrodes such as reference and working electrode. The reference electrode possesses the fixed potential whereas working potential exhibits variable values of electric potential as per the analyte concentration. The electrode of this biosensor may be selective for ions /gases such as H+, Na+, K+, Ca++, NH4+, NH3, F-, I- and CN-. Glucometer is an instrument of potentiometric type of biosensor which calculates the glucose level in blood sample (Pisoschi 2016).

**4.6 Amperometric Biosensor**

This biosensor measures the movement of electron generated during oxidation-reduction reaction when between two electrodes, a constant potential is applied. This biosensor is fast and very sensitive in terms of its ability to sense even 10-9 M analyte concentration (Sadeghi 2013).

**4.7 Conductometric Biosensor**

This biosensor uses a conductive polymer like polyaniline, polypyrrole, polyacetylene etc. for labeling of bio-receptors to generate electrical signal for detection of a biological signals. Muhammad-tahir and Alocilja (2003) has designed a conductometric biosensor using polyaniline labeled polyclonal antibodies for the detection of *E. coli* O157:H7 and *Salmonella* sps. with the detection limit 79cfu/ml and 83 cfu/ml respectively ([Dzyadevych](https://www.sciencedirect.com/science/article/pii/B9780857095015500064#!) & [Jaffrezic-Renault](https://www.sciencedirect.com/science/article/pii/B9780857095015500064#!) 2014).

**4.8 Calorimetric Biosensor**

This biosensor measures change in heat generated during exothermic enzyme catalyzed reactions. It uses two thermistors to develop electric signal after measuring the difference in heat generated/lost between reactants and products. This biosensor requires a heat insulated jacket to prevent heat loss but this necessity makes it bulky. The sensitivity of this biosensor is not as high as it requires the accuracy in thermistors. This biosensor also requires large volume of sample for detectable change in temperature (Danielsson 1990).

**5. Application of Biosensor in Milk and Certain Selected Packaged Milk Products**

**5.1 Detection of Antibiotics and Drugs**

Several veterinary drugs are used against bacterial infections in livestock, hence in packaged milk and milk products their residues might occur and cause human illness upon consumption such as allergy, microbial resistance etc. Tetracycline is an economically valuable, natural and broad-spectrum polypeptide antibiotic which inhibits the protein synthesis of target pathogens. It is used to treat various bacterial infections not for treating viral infections like flu or cold. It can be produced by fermentation using species of *Streptomyces*. It is widely utilized as growth promoter in animals hence there occurrence in milk, meat, eggs and chicken is obvious. In order for oral uptake of tetracycline, medical professionals do not prescribe to uptake it with milk and milk products because the calcium of the milk binds with tetracycline and inhibits its absorption in the gut. Therefore, there is an increasing demand of novel technology like biosensor to detect the level of tetracycline in milk and milk products (Chopra and Roberts 2001). Zhang et al (2010) has developed sensitive, inexpensive and reliable DNA aptamers based biosensor for tetracycline detection in milk using modified glassy carbon electrode with the detection limit of 5 min.

Streptomycin, a broad-spectrum antibiotic is used for the treatment of gram-negative infections. Its uncontrolled administration in foodstuffs causes serious illness such as nephrotoxicity and ototoxicity. As per the recommendation of European commission, the maximum permissible limit of streptomycin in milk should not be more than 200μg/kg. Therefore, for safety point of view, detection and quantification of streptomycin in milk is in urgent demand. Ramezani et al (2017) has developed an electrochemical aptasensor for the detection of streptomycin in milk with the detection limit as low as 11.4nM. In the continuation, Ramezani et al (2017) also designed fluorescence quenching and colorimetric aptasensor for the detection of streptomycin in the milk with the detection limit as low as 56.2 and 108.7nM, respectively.

**5.2 Detection of Pesticides**

Contamination of milk with different neurotoxic pesticides is one of the major serious health issues. The most commonly pesticide group, organophosphorus (OPs) infects milk and milk products via several ways. The pesticides are used directly on domestic animals for ecto-parasites control. The cattle absorb these pesticides by inhalation, ingestion and from skin and thus contaminated milk are secreted by the animals. The pesticides affect normal functioning of nervous system due to its binding with acetylcholine esterase enzyme resulting in weakness and paralysis of muscles (Fagnani et al 2011). Therefore, European Union has recommended very low limit (10µg/kg) of pesticides in baby food. Mishra et al (2012) has developed a screen printed carbon electrode in an automated flow based biosensor by utilizing genetically engineered acetylcholine esterase with the detection limit 5×10−12 M, 5×10−9M and 5×10−10M respectively for toxicity detection in fat containing milk by neurotoxic pesticides organophosphates (chlorpyriphos-oxon, ethyl paraoxon and malaoxon) in less than 15 minutes.

**5.3 Detection of Heavy Metals**

Heavy metals impart serious threat on human health through the daily diet beyond certain concentrations of regulation level. Even their trace amount if present in food can cause health issues. In industrial regions heavy metals such as lead, cadmium, zinc, copper and selenium readily migrate through milk and milk products. Cadmium and lead affect kidney and nervous system whereas copper, zinc and selenium are essential for normal functioning of human health. The detection of these heavy metals in milk and food is very challenging issue as far as our health is concerned. Traditionally, atomic absorption spectroscopy, optical emission spectrometry, microarray are applied for heavy metals detection but lengthy sample treatment process such as desalting, filtration and concentration are their limitations. Therefore a rapid, reliable and sensitive procedure for heavy metals detection in milk and food is needed to be applied. Urea biosensors can be used for heavy metals detection. Heavy metals inhibit urease activity. This inhibition is an index and based on the interaction of heavy metal ions with thiol ormethylthiol group of urea (Singh et al 2008). Kumar et al (2017) has developed specific recombinant biosensor in the host *E. coli* DH5α to detect lead, zinc and cadmium in 15 min of response time by expressing gfp gene encoding for green fluorescent protein of *Staphylococcus aureus* plasmid pI258.

**5.4 Detection of Toxins**

Zearalenone is a mycotoxin which is found as a common contaminant in milk. It causes mycotoxicoses upon consumption of mycotoxin contaminated milk. Valimaa et al (2010) has utilized genetically modified strain of *Saccharomyces cerevisiae*as biological active elementin microbial biosensor for the detection of zearalenone in milk for its qualitative analysis.

**5.5 Detection of Pathogens**

*Salmonella* is gram-negative enterobacteria. Its species *S. enterica, S. paratyphi* and *S. bongori* are causative agents of food borne diseases in human such as diarrhoea, vomiting, gastroenteritis and typhoid. Its detection in food and milk is an urgent need for human safe health. For detection of *Salmonella* in milk, Liebana et al (2009) has designed an immunosensor by making a sandwich with two (one HPR labeled and other associated with magnetic work sensor) polyclonal anti-Salmonella antibodies as bioreceptor. This biosensor could be able to detect 7.5×103cfu/ml in 1/10 diluted milk. The researcher has also developed another enhanced biosensor by using DNA as biological active compound coupled with HPR enzyme for the detection of *Salmonella*. This biosensor could be able to detect 1 cfu/ml of food borne pathogenin the sample. An antibiotic conjugated magnetic nanoparticle (AbMNPs) has been used to detect *Salmonella* in milk with the sensitivity 100 cfu/ml (Joo et al 2012).

*E. coli* O157:H7 is a toxin producing serotype of *E. coli* which causes hemorrhagic colitis, stomach cramps, hemolytic uremic syndrome and anemia. This serotype is transmitted to human beings by the consumption of raw milk and meat products. Mao et al (2006) has developed a DNA biosensor using thiolated and specified ssDNA probe for eaeA gene and streptavidin-conjugated MNPs for detection of *E. coli*O157:H7 with the detection limit 2.67 x 102 CFU/mL. For the same objective, Chen et al (2008) has designed piezoelectric biosensor using GNPs and thiolated ssDNA probe for eae A gene for detection of *E. coli* O157:H7 with the detection limit 1.2 x 102 CFU/mL. An electrochemical immunosensor has been designed by Cho et al (2008) by immobilizing anti-*E. coli* O157:H7 antibody on PNse SPCE (peptide nanotubes on SPCE). Furthermore, Maurer et al (2012) has detected *E. coli* using a nanobiosensor and RNA coated GNPs conjugated with carbon nanotubes. Branen et al (2007) has detected *E. coli* O157:H7 and *S. enterica*sero var *typhimurium* in milk by designing a fluorescence biosensor using antibody-MNPs conjugate with the detection limit 2.4x103CFU/mL and 1.9x104 CFU/mL, respectively.

*Mycobacterium avium* sps. *paratuberculosis* (MAP) is the causal bacterium of Johne's disease in cattle. For the detection of MAP in milk, Kaittanis et al (2007) has developed a magnetic nanosensor by conjugative super paramagnetic iron oxide nanoparticles (SPIONs) with anti-MAP antibodies. This nanosensor has measured the MAP in dose dependent manner.

**5.6 Detection of Biomolecules**

The concentration of lactate as metabolite has been widely used as a key parameter in many food and fermentative industry for food analysis. The lactate is generally used as a specific indicator of bacterial fermentation and its presence indicates the freshness, stability, shelf-life, flavor and quality of food products. Fermented milk products produce lactate and its on-site detection could be performed by enzyme based lactate biosensors. In the L-lactose biosensors, two enzymes commonly used as biological recognition elements are L- lactate oxidase (LOD) and L- lactate dehydrogenase (LDH). L-LOD oxidizes L- lactate into pyruvate and electrochemical hydrogen peroxide which either get reduced or oxidized to yield a current proportional to the concentration of L-lactate. LDH catalyzes L-lactate into pyruvate and NAD to NADH. NADH is oxidized on the electrode surface in the presence of applied potential and obtained oxidation current indicates the equal proportion of L- lactate in the solution (Rathee et al 2016). Eshkenazi et al (2000) has developed a multi enzymatic amperometric bioassay for lactose detection in fresh raw milk in order to be used this biosensor as an economical on-line measurement of lactose in milk parlors.

Bovine serum albumin (BSA) is a component of whey protein systems of cows’ milk. It comprises of 1.5 % of total milk protein. BSA has been shown as potential auto immune trigger of insulin dependent diabetes mellitus. Therefore, consumption of bovine milk and pediatric formula milk may cause hypersensitivity and allergy to human population which are susceptible to BSA (Villa et al 2018).

Lactulose is a disaccharide of galactose and fructose. It is normally not found in milk but when heat treatment (more than 70oC) is provided to milk in alkaline solution, due to isomerization of lactose, lactulose is produced. As it is initially not present in milk but after heat treatment lactulose is formed therefore it becomes the analytical indicator of heat treatment in UHT milk. Moreover, it is useful to treat chronic constipation and also enhances growth of *Bifidobacterium* in human gut. Its upper threshold value has been proposed as 60mg/100ml of UHT milk to avoid excess heating. Therefore, to calculate the lactulose value in heat processed milk, Moscone et al (1999) has developed enzyme based biosensor for detecting lactulose in the milk by using β-galactosidase enzyme for hydrolyzing lactulose into galactose and fructose and fructose dehydrogenase was used to measure the amount of fructose produced.

In the cheese manufacturing, rennet (chymosin) treatment of cheese releases whey containing glycomacropeptide (GMP). It is produced by cleavage of kappa casein. It is biological active glycopeptides possessing emulsifying property, wide range of pH solubility, foaming and gel formation ability. It is used as one of the ingredients of functional food. It plays as important indicator of sweet/rennet cheese whey adulteration in milk and milk products. Adulteration by cheese whey is very cost effective than adding milk and it does not affect the sensorial properties of the milk and its products as well. Adulteration of milk and milk products has been a major issue for both consumers and manufactures of dairy industry. Therefore for detection of adulteration in milk and milk products by cheese whey, several efforts have been carried out in biosensor technology using GMP as indicator molecule. To detect whey addition in milk and milk powder Haasnoot et al (2006) has designed a SPR biosensor (Surface Plasmon Resonance) by immobilizing monoclonal antibodies against kappa casein for detecting GMP as test analyte. L and D amino acids like leucine, phenylalanine, glycine are found in several fermented products such as yoghurt, cheese etc. These amino acids are indicators of contamination of microbes and age of fermentation of products. Moreover, they also contribute for some health issues such as abnormality in amino acid metabolism in human. Therefore, for the detection purpose, Sarkar et al (1999) has developed a screen printed amperometric biosensor for rapid detection of L and D amino acids using L and/or D amino acid oxidase enzyme.

**5.7 Detection of Additives and Allergens**

For enhancing milk yield, animals are treated with recombinant bovine somatotropin (rBST). But this treatment in animals stimulates the production of a polypeptide, IGF-1. It is Insulin like growth factor which is produced from liver and other tissues in body. IGF-1 causes serious health issues as it is a potent carcinogen and having proliferation and differentiation ability. Therefore detection of milk and milk products for the quantitative measurement of IGF-1 is needed to be performed. Hence, Guidi et al (2001) has developed SPR-biosensor using polyclonal anti IGF-1 antibodies for cows’ milk sample analysis.

**5.8 Detection of Adulterants**

Nowadays several adulterants are added in milk to make it synthetic such as urea, refined oil, detergent, melamine, caustic soda, starch etc. which leads to the health hazards to the milk. Urea is a normal constituent of milk. It represents 55% of total non-protein nitrogen of milk. Urea is commonly added as adulterant as it is cheap and rich in nitrogen content. Urea biosensor is advanced technology for monitoring urea content as adulterant of milk during manufacture and quality control. It is an essential approach because above the cut-off limit in milk, urea causes indigestion, acidity, ulcer, cancer and kidneys malfunction. The amount of urea present in the milk sample can be determined by observing the change in pressure caused by carbon dioxide produced in a sealed cell with citric acid.This carbon dioxide is produced by carbonate ions released by the hydrolysis of urea with urease. This pressure based assay is simple, inexpensive and accurate (Jenkins et al 2000). Verma and Singh (2003) have also developed urea biosensor with the response time of 2 minutes. This biosensor has been designed by physical adsorption of urease yielding biomass over Whatman filter paper and coupling with ammonium ion selective electrode of transducer. Renny et al (2005) has developed biosensor for urea detection (0-10 mg/dl) within 3 minutes of response in diluted milk. The limitation with this biosensor is that milk has to be diluted to overcome the disturbances caused by solids of milk. Ramesh et al (2015) has also designed urea biosensor by immobilizing *Arthrobacter creatinolyticus* urease on modified PAN membrane followed by integrated with electrode. By this biosensor, 1-100mM urea concentration could be determined. The immobilize enzyme could be stored at 4 °C for 70 days. The biosensor was found with 13 times reusability efficiency as well without any loss in enzymatic activity.

Melamine (C3H6N6) is chemically known as 1, 3, 5-triazine-2, 4, 6-triamine. It is produced from dealkylation of the pesticide, cyromazine. This pesticide is used as insect growth control. EU does not recommend use of cyromazine on the milk producing animals in order to prevent its transmission to human upon milk consumption. Adulteration of melamine in milk and milk related products to deliberately enhance the protein content is a serious issue because melamine affects kidney and urinary tract functioning. Therefore immune-biosensor has been designed to detect melamine in infant formula milk and infant liquid milk samples by immobilizing poly clonal antibodies against test analyte (Fodey et al 2011).Maltose, sucrose, glucose, malto dextrin, and Phosphate test is also being used for detection of adulteration.

**6. Recent Advances in Biosensor Technology**

Nano-biosensor technology’s the combination of science and technology at nanoscale (approximately 1 to 100 nm) to convey information of nanoparticles to macroscopic world as quantifiable electrical signal. It has shown great attractive prospects in every step of food industries from production, processing, safety, packaging, transportation, storage and up to delivery. Nanomaterials-based biosensors are playing an important role in detection and analysis of food contaminants as these possess high sensitivity and specificity in target recognition. Nanomaterials improve electrochemical, mechanical, magnetic and optical properties of biosensors. The nanoparticles are stronger, lighter, durable and highly efficient and allow the placement of tiny structures with precision and simplicity. Also it is of low cost and takes less analysis time to readout the signals. Several nanotechnology based biosensors such as magnetic particles, gold nanoparticles (NPs), quantum dots, nano rods and carbon nanotubes (CNTs) have been developed for detecting pathogenic microflora and their toxins (Kumar et al 2020). The pathogenic microfloral identification has been performed by Kaittanis et al (2010) by using PPE-AuNPs conjugate (Poly paraphenylene ethylene-gold nanoparticles). Similarly, rapid detection of pathogenic *Salmonella* has been carried out in milk using super paramagnetic Ab-MNPs (Antibody conjugated Magnetic NPs) and optical nanocrystal probes by Joo et al (2012).Bio-functionalized MNPs and anti-microbial antibody has also been utilized in milk to detect *E. coli* in low detection limit and short detection time (20 CFU/ml and 1 hour, respectively) by using BMNPs-Ab conjugate, ATP bioluminescence and external magnetic field (Cheng et al 2009).

**7. Conclusion**

Numerous development and research innovations to devise ultrasensitive biosensors have played an important role in milk and food packaging analysis. The biosensor based analysis are user friendly, simple, sensitive, reliable, portable and economic with low detection limits in short time period. However more research is needed regarding slow dynamic response, biocompatibility and the technical problems. Moreover, various health and safety issues are associated with the use of nanoparticles in biosensor technology. It causes serious illness to human body. Carbon nanotubes can cause lung infection. Some political and social issues and loss of jobs are also associated with this. Nevertheless, with the advancement of nanotechnology in biosensor, nanoparticles are providing suitable support as working electrode for commercialization.

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