JUST AGRICULTURE
multidisciplinary e-Newsletter

Role of Colorimetric Biosensor in Detection of Milk Adulterants

Sakshi Goyal*[1], Kajal Kumari[1], Sonu Maan[1], Pooja Sharma[2] and Sushil Nagar[1]

[1] Department of Biochemistry, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar, Haryana, India (125004)

[2] Department of Chemistry, College of Basic Sciences and Humanities, CCS Haryana Agricultural University, Hisar, Haryana, India (125004)

ARTICLE ID: 49

Introduction

Milk is important source of protein and calcium in human diet and with increasing demand it had become vulnerable to economic adulteration during processing and supply chain. Adulterants are difficult to detect by consumers and thus necessitating the requirement of rapid, accurate and sensitive detection. There are wide range of adulterants which are found in milk such as melamine, urea, formalin, potassium dichromate, detergent, vegetable oil and benzene. Milk being an excellent medium for microbial growth is routinely found to be contaminated with *Listeria monocytogenes, Escherichia coli, Salmonella, Staphylococcus aureus.* The presence of these pathogenic bacteria may prove to be fatal for humans. Pesticides and antibiotics are another major contaminant which get indirectly incorporated into milk and milk products. Consumer being unaware about these adulterants and contaminants is at higher risk in compromising their health. This scenario necessitates the development of devices or methodsto detectadulterants and contaminants which are user-friendly, cost-effective and time-saving. Gold nanoparticles are used in colorimetric sensing due to their simple preparation, easy functionalization, real-time, sensitive detection, and direct observation by naked eyes without use of sophisticated instrumentation. Gold nanoparticles can be modified to increase the sensitivity for detection. In this way, different methods could be utilized for easy detection of milk contaminants and adulterants.

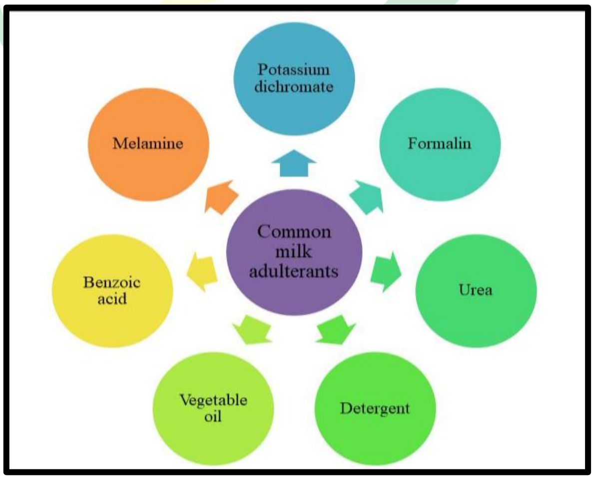
Keywords- Milk, Adulterants, Contaminants, Biosensor, Colorimetric detection

Introduction

Biosensor is an analytical device which incorporates a biologically active element with an appropriate physical transducer to generate a measurable signal proportional to the concentration of chemical species in any type of sample. It may employ a biochemical reaction to detect the presence of chemicals. It is composed of a bio-element function as a receptor that recognizes the target analyte and a transducer that converts the recognition into a signal. Adulteration is a legal offence, when the food fails to meet standards set by the government due to the addition of substances that degrade the quality of food when added to it. Food is said to be adulterated if any of the following happened to it: Any substance which decreases its value or makes it injurious; less expensive or inferior substances are substituted wholly or in part; any valuable constituents have been subtracted either entirely or in part and no matter what the reason is, if its quality is below the standard. Food in general is adulterated for different reasons. Some organizations or individuals in food supply chains are adding adulterants to foods for improving the appearance to get better price by cheating buyers of the products. Food can also adulterated for increasing volume, preserving or others. Generally, adulterations can be intentional and unintentional.

Milk adulteration

The fraudulent practice of milk adulteration is on the rise, which is making people apprehensive about the purity and quality of milk. A variety of milk adulterants are added into milk from economic point of view, but the effects they cause are deleterious (Nagraik et al., 2021). Melamine is a nitrogen rich compound which is added to milk in order to spike the natural protein content of the milk (Liu et al., 2012).



Some amount of melamine is expected to be present in milk because it acquires from plastics used for milk packaging and use of nitrogen rich fertilizers. FSSAI (Food Safety and Standards Authority of India) has set melamine content ranging from 0.5 ppm to 2.5 ppm in different milk and food products (Lawley et al., 2013). Urea is another component that is added exogenously into milk, though some amount of it exists naturally. The permissible limit of urea present in milk set is 70 mg/ml by FSSAI (Sharma et al., 2012). Formalin is used as a food preservative and is also added into milk, but it is associated with renal problems, as reported in the consumers of formalin adulterated milk (Singh et al., 2015). There are various kinds of pathogenic bacteria which grow in milk including *Lactococcus, Lactobacillus, Streptococcus, Staphylococcus and Micrococcus* spp. (Afriat et al., 2020). There have been reports of milk adulteration with other preservatives such as potassium dichromate, benzoic acid, hydrogen peroxide, salicylic acid etc. putting common people's health at stake. Another component that has been tagged as a milk adulterant is ammonium sulphate, which is added to milk to falsely maintain the required density. The addition of detergents in milk to impart it rich frothy appearance and the vegetable oils are added in milk to replace the natural fat content of milk. Whey protein is also added intentionally in milk to manipulate the natural protein content.

Detection of milk adulterants

Melamine is the most common adulterant found in milk. Various methods had been developed to detect the presence of melamine and biosensor is one of them. There is development of colorimetric method in presence of melamine which acts as visual method for detection. Melamine, with a chemical formula of $C_3H_6N_6$ (1,3,5-triazine-2,4,6-triamine), is a nitrogen-rich industrial compound which has been widely used in the manufacture of polymer resins, flame retardants, fertilizers and kitchenware. Unfortunately, melamine has been adulterated into dairy products such as infant formula or animal feeds due to its high nitrogen content (66% by mass).Gold nanoparticles are recently used in various processes involving detection of some harmful compounds due their unique chemical and physical properties. These have extremely high extinction coefficient and strongly distance dependent optical properties which make them favorable for colorimetric sensing. These are easy to synthesize, easy functionalization, real time observations and sensitive detection without complicated instrument. The detection using gold nanoparticles does not require any sophisticated techniques. The interaction of melamine and dispersed gold nanoparticles will disturb the electrostatic balance on the surface of particles and may lead to their aggregation, which result in surface plasmon resonance shift with a consequent variation of color change that can be easily observed with naked eyes and UV-visible spectrophotometer.Label free detection of melamine was carried out using aptamer based biosensor in raw milk. Microarrays were utilized for selection of aptamer so as to obtain aptamers having high affinity against melamine. DNAzyme was conjugated with selected aptamer for detection of melamine (Kaneko et al., 2018).

Conclusion

Milk being an excellent medium for microbial growth gets easily contaminated with various pathogenic bacteria. Milk is consumed as such without any processing, which further necessitates stringent techniques for it's quality check. Biosensors are rapid, cost-effective, user friendly methods which are utilized for detection of various adulterants and contaminants in milk. The colorimetric antibody-based system was developed to detect contaminants of milk. Further studies are required for development of specific and sensitive biosensor.

References

Afriat, R., Chalupowicz, D., & Eltzov, E. (2020). Development of a point-of-care technology for bacterial identification in milk. *Talanta*, 219, 121223.

Kaneko, N., Horii, K., Akitomi, J., Kato, S., Shiratori, I., & Waga, I. (2018). An aptamer-based biosensor for direct, label-free detection of melamine in raw milk. Sensors, 18(10), 3227.

Lawley, T. D., & Walker, A. W. (2013). Intestinal colonization resistance. *Immunology*, 138(1), 1-11.

Liu, Y., Todd, E. E., Zhang, Q., Shi, J. R., & Liu, X. J. (2012). Recent developments in the detection of melamine. *Journal of Zhejiang University Science B*, 13(7), 525-532.

Nagraik, R., Sharma, A., Kumar, D., Chawla, P., & Kumar, A. P. (2021). Milk adulterant detection: Conventional and biosensor based approaches: A review. *Sensing and Bio-Sensing Research*, 33, 100433.

Sharma, R., Rajput, Y. S., & Barui, A. K. (2012). *Detection of Adulterants in Milk: Laboratory Manual*. India: NDRI.

Singh, P., & Gandhi, N. (2015). Milk preservatives and adulterants: processing, regulatory and safety issues. *Food Reviews International*, 31(3), 236-261.