UNLOCKING PRECISION: A DIVE INTO ELISA METHODS AND SIGNIFICANCE IN ENSURING FOOD SAFETY AND QUALITY

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ABSRACT

Enzyme-Linked Immunosorbent Assay (ELISA) procedures utilize the most sensitive and specific immunological reactions, hence they are important tools in food analysis. These versatile techniques are good at detecting specific components in food, such as natural compounds, pesticides, therapeutic drugs active microorganisms and toxins. ELISA can be easily used as an analytical tool for detecting and measuring food production constituent-related quantities ensuring safety of food. In the post-production stage, it is essential to verify food products in order not misrepresent them by using labels. Thus, ELISA is a powerful tool for validating food adulterations whereby consumers are better placed to make proper dietary decisions. ELISA techniques are widely used in the food industry, including indirect, sandwich and competitive ELISA using both polyclonal and monoclonal antibodies. ELISA appears as an alternative method in food analysis, thus providing a path to minimize the reliance on advanced expensive and time-consuming systems without sacrificing sensitivity or reliability. The main objective of this chapter is to discuss the basic aspects of ELISA technique and its utilization in food analysis that has played a crucial role in ensuring quality control and safety for foods being produced by different companies.

Keywords: ELISA, Immunological reactions, Food analysis, Adulterations, Antibodies, Quality control.

INTRODUCTION

One of the major threats to human existence in this world is food safety that not only has economic implications, but it also endangers consumers' health[1]. Food analysis is highly important for identifying and characterizing different components, which

provide valuable insights into factors affecting food properties. These include microorganisms (both pathogens and spoilage microbes), food toxins, beneficial constituents as well impurities[2]. Sensitive testing for food quality ensures the systemic governance of safe and nutritious foods from which consumers can choose by reading about their qualities on labels[3]. Food products are verified at various stages of making, from harvesting raw material to preprocesses processing storage and consumption. Specificity, sensitivity, reliability, simplicity per time and costs of analytical techniques should be the determining factors[4]. Although traditional chromatographic approaches are procedurally demanding and lack sensitivity, the genetic test is extremely specific but stipulated with costly considerations also requiring expertise. Immunoassays mainly ELISA are presented as the most feasible variants for food analysis in terms of simplicity, fast diagnosis and their low-cost apparatus design along with high specificity[5]. The indirect, competitive, and sandwich ELISA variants are commonly used in food testing[6].

The indirect ELISA consists of two antibodies, antigen-specific and enzyme coupled with the second one producing a signal upon binding to fluorogenic or chromogenic substrate[7]. In sandwich ELISA, the antigen is bound between two antibodies capture and detection with subsequent enzyme-coupling of the latter to initiate biochemical reactions[6]. Competitive ELISA is an assay that measures the concentration of antibody or antigen by detecting interference in a predetermined output signal framework[8]. This comprehensive review briefly outlines the principles of ELISA technique, types and its uses in food testing involving detection for useful or harmful ingredients, quality control confirming constituent properties during their use as a production material, determination of spoilage microorganism's concentration in storage processes to prove authenticity by detecting adulterants.

OVERVIEW OF ELISA METHODOLOGY:

PRINCIPLE:

ELISA is an technique, which was invented by Peter Perlmann and Eva Engvall in 1971 at the University of Stockholm, Sweden [9]. This method is very common in quantifying substances like peptides, antibodies, enzymes and hormones present within different samples (Fig.1). ELISA, on the other hand, serves as a plate-based method with an antibody enzyme conjugate interacting with a chromogenic substrate to create an adaptable system for scientific research[10].

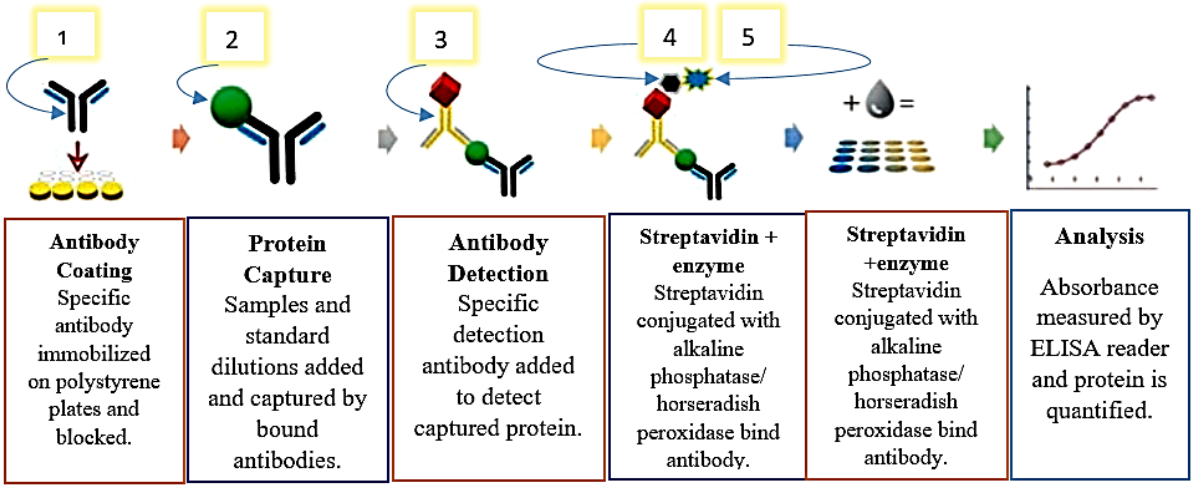

Fig 1. Show the working principle of ELISA. 1. *Coating antibody,* 2. *Antigen (Sample & standard), 3. Specific biotinylated detection antibody, 4. Streptavidin conjugated with alkaline phosphatase or horseradish peroxidase, 5. Colorimetric substrate.*

A significant application of ELISA is characterizing specific peptides in bacterial, flavor chemical compound and other food content. This characteristic makes ELISA an effective tool for judging the quality, safety and even authenticity of food products[10]. The usefulness of this technique also extends to its capability in analyzing a wide range of materials that are crucial in understanding the composition profiles for samples. ELISA uses different enzymes such as AP, BG and HRP which can be used for generalization of various analyses. The technique most commonly uses 96-well polystyrene plates, each containing a different test sample. For accuracy 96 samples include a positive control and negative sample for comparative studies. It starts with an antigen or antibody on the solid phase binding to capture antibodies and antigens in serum. Further procedures include careful rinsing with successive buffers in order to get rid of the unbound antibodies or antigens [11].

In the following step, secondary antibody coupled with an enzyme such as alkaline phosphatase or peroxidase is applied to each well. After incubation, the secondary antibodies are removed through washing. The application of an appropriate substrate makes the enzyme in the well start a biochemical reaction, generating its specific color. This color's intensity, spectrophotometrically determined at the wavelength of 450 nm correlates to optical density. This optical density can be used as an estimation of the concentration, allowing us to determine how much antigens or antibodies are present in a specified sample. The ELISA is a potent and versatile technique for research in the fields of immunology as well as food analysis[12].

TYPE OF ELISA:

ELISA is a widely acknowledged method for detecting multiple targets. Its simplicity and quick results make it a popular

choice in diagnostic and research contexts. As the name suggests, ELISA incorporates enzymes and specific interactions between antigens and antibodies. The technique operates through four main types: direct, indirect, sandwich, and competitive, each serving distinct purposes in analysis and detection processes[13]. Let's explore each of these types individually.

DIRECT ELISA:

In direct ELISA, an enzyme-labeled primary antibody is used and this obviates the use of a secondary antibody (Fig 2). The target (antigen) is immobilized to an ELISA plate or solid surface and the primary antibody-enzyme conjugate "directly" recognizes it. Secondly, the enzyme that is coupled to primary antibody reacts with its substrate producing a detectable signal whose magnitude can be determined. Hereby, the target antigen is revealed[13],[14].

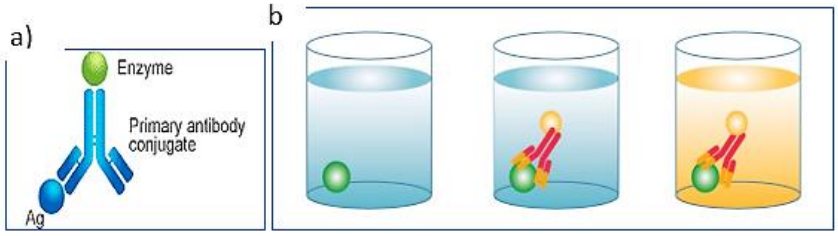

Fig 2. Illustration of direct ELISA, a) structure of direct ELISA. b) working process of direct ELISA.

INDIRECT ELISA:

In the indirect ELISA, a primary antibody and secondary one are used. In this situation, the first antibody is not labeled with enzyme (Fig 3). On the contrary, a secondary antibody has an enzyme label[15].

The primary antibody binds to the immobilized antigen on plate, and then enzyme-linked secondary antibodies react with it. Finally, secondary antibody enzyme produces a visible reaction signal and quantifiable with its substrate[15].

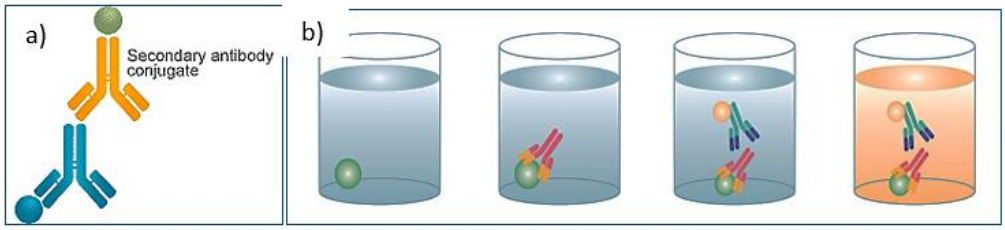

Fig 3. *Illustration of indirect ELISA, a) structure of indirect ELISA. b) working process of indirect ELISA.*

SANDWICH ELISA:

In the sandwich method, which is the most common ELISA method, an antigen is placed between two specific antibodies. In fact, in this technique, first the antibody connects with a solid

phase[16] (Fig 4). A certain amount of the sample to which the antigen is attached is added after washing the secondary antibody labeled with the enzyme, which reacts with the remaining antigens and then binds to the fixed antibody. This technique is very simple. And it is associated with increased specificity and sensitivity[17].

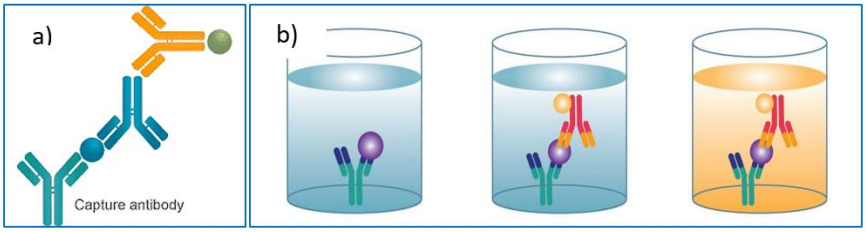

Fig 4. *Illustration sandwich ELISA, a) structure of sandwich ELISA. b) working process of sandwich ELISA.*

COMPETITIVE ELISA:

Competitive or inhibitory ELISA are other common types of this technique. In the competitive method, it is based on the competition of two antigens or two antibodies to bind the ligand with a limited amount[12] (Fig 5). During incubation, the labeled antigen competes with the standard or non-labeled test antigen, after incubation, the reaction wells are washed with a suitable buffer to remove free reactants. Then a substrate solution is added, which produces a colored product after being broken down by the enzyme in the compound[18]. The major advantage of competitive ELISA essentially lies in the higher sensitivity to variations within mixture antigen complex, especially where specific detection antibody is present relatively low quantity. Therefore, as both the analytes are introduced at a time it is called ‘Competitive’. On the other hand, when inhibitory competition occurs, in which initially there is an addition of analyte that after incubation period has added labeled one then method might be termed as inhibitory[19].

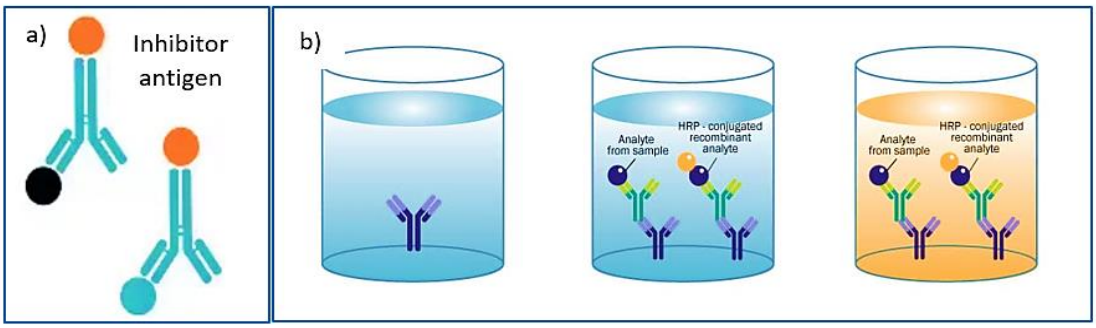

Fig 5. *Illustration of competitive ELISA, a) structure of competitive ELISA. b) working process of competitive ELISA.*

COMPARISON OF FOUR TYPE OF ELISA:

Still, now we understand how the four most common ELISA work but from a point of view of sorting out proper one

for your experimental process. To answer the question, one should know what are advantages and disadvantages of each type ELISA[20].

Table 1. Comparison of four type of ELISA according to their advantages and disadvantages.

ELISA	Advantages	Disadvantages	Ref
Direct ELISA	1. It is simple protocol, time-saving and reagents saving. 2. No secondary antibody cross-reactivity.	1. High background. 2. Because a secondary antibody is not needed, there is no signal amplification. 3. Lack of flexibility, because the primary antibody needs to be labeled.	[18]
Indirect ELISA	1. Because one or more secondary antibodies can bind to the primary antibody, therefore signal amplification. 2. High flexibility, as a single secondary antibody can be used with different primary ones.	1. Complex protocol rather than direct ELISA. 2. Cross-reactivity from secondary antibody.	[21]
Sandwich ELISA	1. High flexibility. 2. High sensitivity. 3. High specificity as various antibodies are targeted to the same antigen for diagnosis.	1. In addition, the antigen that serves as a selectable agent must be big enough such that two different antibodies can target it at distinct epitopes. 2. The problem arises when trying to find two different antibodies that recognize distinct epitopes on the antigen of choice and works well in a sandwich configuration.	[18]
Competitive or Inhibitory ELISA	1. High flexibility. 2. High sensitivity. 3. Most suited for the identification of small antigens even with low concentrations.	1. Relatively complex protocol. 2. Requires inhibitor antigen.	[15]

STEPS TO PERFORM THE ELISA TEST:

The ELISA test is performed with various methods, which are generally divided into two categories: direct and indirect ELISA. In the direct method, the desired antigen or antibody is directly coated on the surface of the solid phase, and then the antibody or its labeled complementary antigen is added to the system. By analyzing the produced signal, it is possible to find out the presence of the desired antigen or antibody in the sample. This method does not have much diagnostic value and is mostly used

for research purposes. In the indirect method, diluted serum is added to the final antigen coated in the solid phase, then the sample is added to it, and after the passage of time in the greenhouse and a washing step, the anti-human globulin is labeled. This method is used to determine the antibody by adding an enzyme to a specific well or by titrating the antibody in the serum[22]. Based on this, the steps of performing the ELISA test are:

1- Coating, which means the absorption of an antigen or antibody It is a body with solid surfaces.

2- Adding samples to be tested.

3- Sufficient time has passed to carry out the reaction, which is called greenhouse reaction of sugars.

4- Washing by ELISA detergent solution, in order to separate bound and reacted reactants from free and unbound reactants.

5- Addition of coupling compounds with enzymes.

6- Again during the incubation period for the reactants.

7- Reusing the ELISA washer for washing.

8- Adding the enzyme substrate to detect the reaction.

9- Greenhouse installation schedule.

10- The whole enzymatic reaction is done by quenchers and optical density reading obtained by ELISA Reader[23].

PERFORMANCE AND IMPORTANCE OF ELISA IN THE FOOD SECTOR:

In today's world, food safety and health, along with valuing food products away from adulteration, are recognized as one of the most important human priorities[24]. For this reason, modern methods of monitoring and quality assessment while strengthening the control of the food cycle from farm and factory to consumption and having scientific supervision to ensure human health and provide reassurance to the consumer. Industrialization of food and agricultural products is the strategy that makes it possible to guarantee continuous access to food[25]. However, chemical, physical and biological risks from harvesting to storage and marketing of products can affect the quality, health and safety of food. Microbial agents, foreign substances (biological, chemical or physical), natural toxins and additional chemicals constitute a threat on food safety and health. Classical approaches rely on chemicals and cultures identified by laboratories to clear the food chain of contaminants while modern technologies such as ELISA contribute in maintaining an ecosystem integrity[26]. Immunological techniques, including those using the ELISA method, allow detecting both chemical

substances and microorganisms in food processing. With the development in Immunological methods can estimate the antigen of some chemical substances, microbial toxins or cell structure, and in pure forms by binding to a protein such as bovine serum albumin, they are injected into laboratory animals such as mice or rabbits to produce specific antibodies[27]. The produced antibodies include polyclonal and monoclonal, respectively, in the first case, they are produced in the serum of the vaccinated animal, which reacts with several antigen sites, in the second case, after the injection of inflammatory B-lymphocytes from mice vaccinated with myeloma cells. Prepared and accompanied by the screening of colonies that secreted monoclonal antibody[28].

In recent years, the identification of different compounds of food products based on protein compounds such as meat, fish and seafood, milk and dairy products based on genetic and immunological techniques such as ELISA due to their simplicity, specificity and sensitivity is widely used in determining their frauds was used[29]; for example, in protein products such as meat, the type of meat, especially the type of minced meat, is very common in dough products, hence the determination of the origin of meat and the absence of fraud in its structure is based on the sensitivity of some people to allergenic compounds, belief-religious reasons. It seems very necessary. Therefore, sensitive and reliable diagnostic tools are needed to determine and identify animal food ingredients, so that for daily tests and large volume of samples, ELISA technique is more specific than genetic methods. As a result, in recent years, ELISA technique is used to identify the origin of animal meat compounds using antibodies against heat-stable animal muscle or serum proteins[15].

In products based on fish or fish meat, cleaning during processing destroys the structural characteristics of the fish and makes it very difficult to distinguish fish species, and it is the basis for importing various frauds and using low-priced fish instead of It provides high price[30]. To prevent such frauds, food laboratories need fast and low-cost techniques such as ELISA to identify the fish species used in fish production. Identification of fish species is often done by culture-independent methods such as PCR and ELISA. But the ELISA method is cheaper and simpler than molecular methods and can be used on a daily basis for a large number of samples. In a research, polyclonal antibodies against the muscle proteins of haddock and sardines have been produced. In addition, monoclonal antibodies have been produced against fish muscle proteins that are able to identify some low-quality species compared to high-quality species, due to the large variety of fish species that are commercially available for identifying species. There are not many commercial ELISA kits available, so the future

development of rapid diagnostic kits in this field can be useful. In dairy products, there is a possibility of fraud in the production of various milk-based products (fig 6), for example, in the production of cheese from sheep's milk due to The price of sheep's milk is higher than that of cow's milk, and to reduce production costs, it is very common to use cow's milk compounds that are rich in antibiotics and are cheaper because of the sensitivity of some people to cow's milk. And the legal and religious rules of distinguishing these compounds are of great importance[31]. Therefore, to evaluate cheeses made from sheep or goat milk, a correct evaluation of the types of milk used in dairy products is needed. Therefore, for legal reasons and to protect the consumer, the type of milk used in dairy products must be correctly determined using sensitive and reliable methods such as ELISA. ELISA technique is widely used to identify milk species due to its rapidity and high specificity and sensitivity[32].

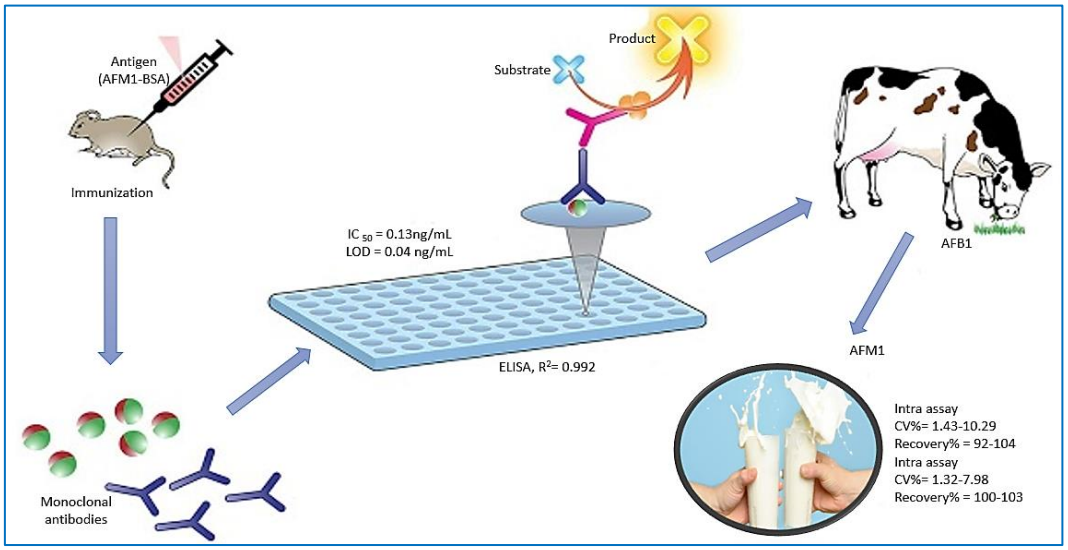

Fig 6. *Demonstrate the identification of the origin of milk and cheese using the ELISA, reproduced by permission from Ref* [33].

In ELISA technique, antibodies are produced for the dominant milk proteins like casein and lactalbumin which could be utilized to analysis as well as identification of counterfeit sheep or buffalo's milk in comparison with cow sparkling by different methods of competitive sandwich type ELISA[31].

In discussion of citrus drinks, systems to ensure security and quality control are very significant since there is a high likelihood of fraud in these products. Adulteration may be diluted with water, usage of cheaper artificial ingredients such as sugars, acids and dyes or by addition citrus fruit pulp mixed peel extracts (Fig 7). For the protection of consumers analytical tools can be used. In order to identify counterfeit juices, trust and sensitivity are required. As a result, grapefruit and orange juice were used to

produce polyclonal antibodies against skin peptides that are employed in the ELISA procedure for identifying counterfeits in juices[33].

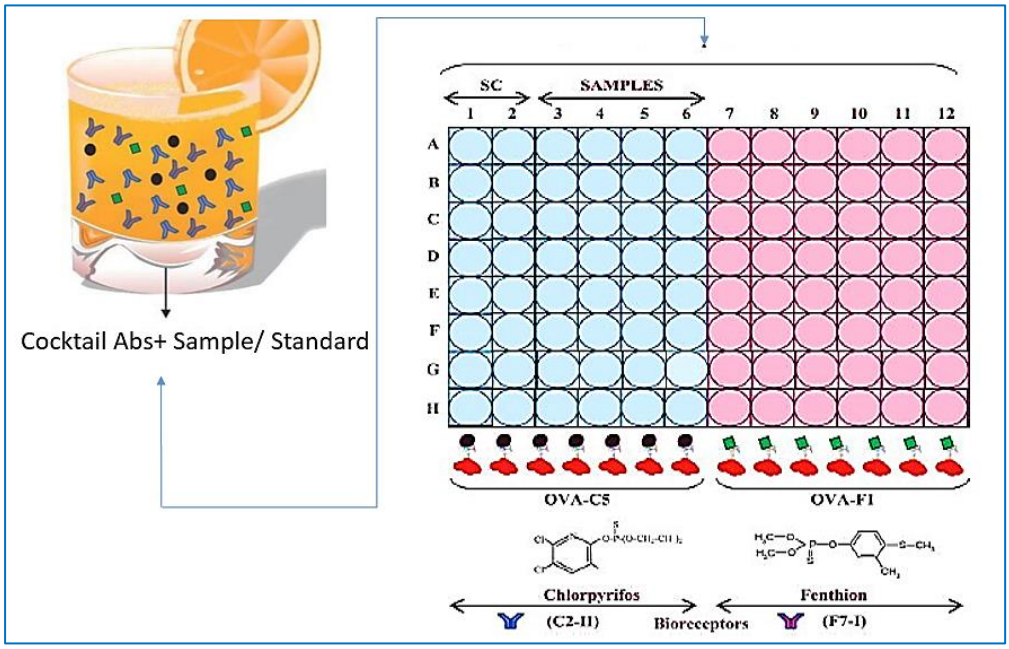

Fig 7. Show the detection of counterfeits in juices with ELISA. Reproduced by permission from Ref[34].

IMMUNOLOGICAL METHOD FOR ESTIMATING AND MONITORING LIVE MICROORGANISMS:

Microbial spoilage (bacterial and fungal) of food is one of the major concerns of food production and processing. Preventing the occurrence of these vulnerable processes will improve access to food and reduce the total price[28]. Immunological methods to identify and estimate the population of active microorganisms Spoilage and ingredients that lead to significant quality problems are used for food production and processing. It can include 20% of food losses. Food spoilage is caused by a variety of molds and bacterial species, immunological methods such as ELISA can control and prevent food spoilage by using early detection of living organisms and population estimation and population monitoring. For example, in molds and yeasts, antigenic compounds in mold extracts can be used for immunofluorescence or ELISA tests to determine the number of molds. Counting mold in tomato puree by ELISA is 100 times more sensitive than chromatography. Unlike conventional culture methods that require several days, the ELISA test only takes 5 to 10 hours. Also, the test time can be reduced to 10 to 20 minutes using the latex agglutinin method. However, the sensitivity of this method will be five to ten times lower than the ELISA technique. Conventional methods still offer certain advantages over safety tests[35]. Many edible mushrooms are identified using light microscopy by examining the morphological characteristics of

spores and mycelium. Since the antibodies created against the fungal hyphae with the culture supernatants do not have specific characteristics to identify the microorganism. Based on this, special antigens such as enzymes, toxins and microbial exopolysaccharides are used as microbial secondary metabolites for antibody production[33].

IMMUNOLOGICAL METHOD FOR MYCOTOXIN ESTIMATION:

Antibody functions as a parasite by attaching to small protein molecules such as mycotoxin. Several researches were done on immunological methods and its application in detecting mycotoxins in foodstuffs, with two techniques; RIA & ELISA being able to quantify levels of mycotoxin-contaminated foods It was observed that the latter excepted a greater sensitivity than RIA for evaluating different quantities of contaminants[36]. and led to improved findings for aflatoxin in corn, wheat, as well as peanut butter. The evolution of monoclonal antibodies to aflatoxins has helped in increasing the sensitivity of ELISA test[37]. In the final absorption column, a monoclonal antibody against aflatoxin was proposed and pure mycotoxins concentration of 0.5 ng were detected using high-performance liquid chromatography This screening procedure just takes 10 minutes[38]. ELISA may be used to detect and track aflatoxin B1 after processing foods or when it is in biological systems using antibodies directed against ADB[39].

CONCLUSION:

ELISA is a fundamental bioanalytical technique employed in detecting and quantifying different substances, specifically effective especially for food analysis. The basic principle of ELISA is founded on the antigen-antibody interaction and using this particular specificity to allow accurate measurement. Two main types, direct and indirect ELISA, offer some degree of flexibility allowing to target different analytical needs whether one wants either the detection a specific analyte or its quantitation. ELISA steps are specific and organized. Starting from immobilization of the target antigen followed by blocking to avoid non-specific interactions, subsequent incubation with primary antibody, and ending in addition of an enzyme linked secondary antibody each contributes towards get accurate and reliable result. The subsequent chromogenic or fluorogenic reaction by the enzyme enables quantification of the target substance, whereby signal strength is a measure that depends on concentration. ELISA assumes a major role in the food industry as it guarantees safe and quality products. The ELISA is one of the most versatile tools used in food analysis as it can be able to detect and quantify specific proteins, allergens. Allergen identification is especially important for this application in the detection of threats to

individuals with specific dietary restrictions. The sensitivity of ELISA makes it possible to detect small concentrations of contaminants thus helping the food industry meet quality standards and ensure safety for consumers. Furthermore, ELISA involves in the surveillance of foodborne pathogens that permit to act on time if contamination occurs. This is critical for the prevention of food-borne diseases and protection of public health. The use of ELISA is an acceptable alternative due to efficiency, speed and cost-effectiveness in the food industry since it can be used for routine analysis. versatility and accuracy make it an essential instrument in the food analysis. Application of this procedure goes beyond traditional laboratory settings, penetrating to the very heart of food safety and quality control. With the rapid advances of technology, digitization leads to new challenges ELISA is always at the forefront by focusing on developing novel techniques that will improve food industry analytical approaches.

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