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Review

Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA)

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Abstract

This work intends to provide an updated and extensive overview on the applications of ELISA techniques for meat, fish and milk species discrimination; fruit juice labeling authentication; genetically modified and irradiated food detection; feedstuffs origin and allergen ingredients identification. These methods have been widely used because they reduce the use of costly, sophisticated equipment and time of analysis and are suitable for routine analysis of a large number of samples. Therefore, ELISA could allow, together with other analytical methods such as DNA-based methods, consumer protection and confidence, and an accurate implementation of the traceability for successful regulatory food controls.
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Keywords: Food authentication; ELISA; Polyclonal and monoclonal antibodies

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1. Introduction

Authenticity testing of food products, such as meat, milk or fish, is important for labeling and assessment of value and is therefore necessary to avoid unfair competition and assure consumers protection against fraudulent practices

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commonly observed in the food industry. Additionally, fraudulent adulteration of food products may be objectionable for health reasons, since consumption of products containing, undeclared constituents may cause problems such as allergy in sensitized individuals (Mackie, 1996).

In this context, food components identification has been mostly performed in the last few years by different techniques. Chromatographic and electrophoretic techniques have proved to be useful in food components identification (Berrini, Tepedino, Borromeo, & Secchi, 2006; Mackie et al., 2000; Mayer, 2005). However, although they are considerable value in certain instances, these methods are not convenient for routine sample analyses because they are relatively costly, time consuming, and complex to perform. Consequently, in the last years the identification of meat and meat-based products, fish and seafood, milk and dairy products, and other foods has been performed primarily by genetic (Bottero, Civera, Anastasio, Turi, & Rosati, 2002; Matsunaga et al., 1999; Terzy et al., 2005) and immunological techniques (Carrera et al., 1997; Liu, Chen, Dorsey, & Hsieh, 2006).

Genetic methods are the most specific and sensitive methods for food components authentication. However, they require expensive laboratory equipment and a certain degree of expertise. As an alternative, immunological assays can be used to reduce the test time and cost. Among these last methods, the Enzyme-Linked ImmunoSorbent Assay (ELISA for short) has been the most widely used technique for regulatory purposes in detecting food authenticity because of its specificity, simplicity and sensitivity, among other advantages (Mackie, 1996). On the whole, ELISA is an immunological technique that involves an enzyme (a protein that catalyzes a biochemical reaction) to detect the presence of an antibody or an antigen in a sample. The two most used variants of ELISA for food authentication are the indirect and the sandwich ELISA. The indirect ELISA utilizes two antibodies, one of which is specific to the antigen and the other of which is coupled to an enzyme. This second antibody gives the assay its ''enzyme-linked'' name, and will cause a chromogenic or fluorogenic substrate to produce a signal. Sometimes this second antibody may be linked to a protein such as avidin or streptavidin if the primary antibody is biotin labeled. In the sandwich ELISA the antigen is bound between two antibodies: the capture antibody and the detection antibody. The detection antibody can be coupled to an enzyme or can bind the conjugate (enzyme-linked antibody) that will produce the biochemical reaction (Goldsby, Kindt, Osborne, & Kuby, 2003).

ELISA tests may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. In quantitative ELISA, the optical density or fluorescent units of the sample is interpolated into a standard curve which is typically a serial dilution of the target (Goldsby et al., 2003).

Nevertheless, the prerequisite for an ELISA is the availability of amounts of antibodies sufficient to detect analytes. Both polyclonal and monoclonal antibodies (MAbs) can be used in ELISA methods for food components identification. Polyclonal antibodies offer a number of benefits such as recognition of a mixture of different epitopes of the antigens, more tolerance to small changes in the nature of antigen, like polymerization or slight denaturation and they are a preferred choice for detection of denaturated proteins. However, they present limitations such as variable affinity, limited production and a requirement for extensive purification procedures to eliminate cross-reactivity for a particular species identification (Harlow & Lane, 1999). In contrast, MAbs are a homogeneous population of antibodies produced by hybridoma technology that have defined biological activity, consistent specificity and their production is not limited (Goding, 1996). Both polyclonal antibodies and MAbs are used in the ELISA variants for food authentication that have been previously described (Harlow & Lane, 1999).

Furthermore, although the great limitation of ELISA methodology is that the target proteins sometimes are denatured during food processing and therefore the target protein epitope may not be present in the condition detectable by the antibodies, this limitation has been solved because of the development of antibodies against thermostable proteins (Ansfield, 1994; Ansfield, Reaney, & Jackman, 2000; Berger, Mageau, Schwab, & Johnston, 1988).

In the last years, advances in ELISA technology have led to rapid development of different commercial immunoassays kits for use in the food and feed industry. The most used immunoassays kits for food components identification are ELISA and lateral flow tests. ELISA test kits may be performed in microtiter plates and immunosticks formats. Lateral flow tests or ''dipsticks'' employ the same immunoassay principles as the ELISA tests but coat the antibodies and other reagents on a nitrocellulose membrane rather than the inside of test wells or paddles and they use colloidal gold, dye, or latex bead conjugates to generate signal rather than enzymes bead conjugates. The simplicity of both type of tests and the short time required for the analysis make them suitable for food screening tests of a large number of samples (Bonwick & Smith, 2004).

On the basis of this information, we report in the present review the ELISA applicability on food authenticity in the last few years. We have described the use of ELISA techniques for meat, fish and milk species discrimination; fruit juice labeling authentication; genetically modified and irradiated food detection; feedstuffs origin and allergen ingredients identification.

2. Meat and meat-based products

Legislative authority establishes that meat products must be accurately labeled regarding species content. Meat species adulteration in ground and comminuted products has been a widespread problem in retail markets. Identifi-

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