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Enzyme Based Biomarkers – Applications in Novel Food Processing and Analysis

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Abstract
In this review content, the enzyme based biomarkers, applications of biomarkers for novel food processing and analysis have been expressed. Nanoscale biosensors incorporating enzymes are being developed for detection of chemical contaminants, allergens and other protein moieties and for food pathogens. Enzymes are also a component in active packaging systems and have been recently incorporated into traceability and authentication tags for high value or frequently counterfeited items.

Keywords: Enzyme, biomarker, novel food processing, quality

Introduction

Recent rapid advances in enzyme based biosensors have improved both process monitoring and control in the food industry. Many new advances in biosensor technology have made rapid detection and differentiation of microorganisms possible this includes advances in microfluidics systems for detection and quantification of viable bacteria and bacterial DNA. The most readily adopted commercial systems in the food industry have been adapted from medical applications and technical platforms from this field. A number of analytical systems for mycotoxins, agricultural chemicals and trace organic contaminants employing metallic nanoparticles for signal enhancement in biosensor detection systems are at the point of commercialization and food industry use. Similarly, enzyme based analytical systems for detection of components responsible for food flavor and aroma are available mostly for quality control and product development and as a replacement for more expensive analytical tests or to reduce requirements for sensory testing. Nanoscale biosensors incorporating enzymes are being developed for detection of chemical contaminants, allergens and other protein moieties and for food pathogens. Unfortunately, advances in the enzyme based biosensors for process control applications are progressing more slowly, but miniaturized flow injection systems for small molecules such as sugars in liquid process and waste streams are commercially used. On a limited scale, predictive models for heat and pressure exposure, predicting energy distribution, and heating uniformity use enzyme based sensors. Enzymes are components in retail 'smart' packaging for predicting thermal abuse. Enzymes are also a component in active packaging systems and have been recently incorporated into traceability and authentication tags for high value or frequently counterfeited items.

Biosensors and Their Efficiencies

Biosensors are a subgroup of chemical sensors serving as components of analytical devices composed of: a biological recognition element such as an enzyme, antibody, receptor or microorganism coupled to a chemical or physical transducer. The most common tranducers used with enzyme based sensors are: electrochemical, mass, optical and to a lesser extent, thermal. Enzyme based sensors for both analytical and process control applications in the food industry are most commonly hydrolases and oxidases.

Oxidases are stable and many oxidases have the advantage of no requirement for coenzymes or other cofactors as part of the reaction system. For example, glucose detection using an entrapped or immobilized glucose oxidase electrode are widely used. In this case, enzymes are physically entrapped in close proximity to a tranducer or chemically immobilized to it. Colorimetric detection of sugars and sugar alcohols, ethanol, peptides employ immobilized enzymes as components of biosensor based systems. Detection systems may be potentiometric, amperometric, optoelectric, calorimetric or piezoelectric and, if possible, commercially available enzymes are used to simplify construction and reduce cost. These enzyme based systems are the basis for nanoscale sensors describe in Figure 1 with microfluidic devices potentially have substantial commercial potential in the food industry.

Recent advances have made it possible to detect aroma and flavor quality properties of fermented beverages, roasted coffee and spices using biosensors of various types.
Real time sugar detection (sucrose, glucose, fructose, maltose) to levels of 0.5 mM using immobilized enzyme detection systems are feasible

for fermentation mixtures, fruit juices and purees and utilize an immobilized glucose oxidase electrode with zinc oxide: cobalt nanocluster based thin film sensors.

Sensitive detection systems for food borne contaminants including pesticides (DDT, parathion, chloropyrifos, paraoxon, and the herbicide 2,4-D to 0.01 mM) and mycotoxins (to 0.001 mM) are available. Incorporation of metallic nanoparticles (gold, silver, zirconium), carbon nanotubes or cadium telluride quantum dots along with appropriate enzymes or haptens enhances sensitivity.

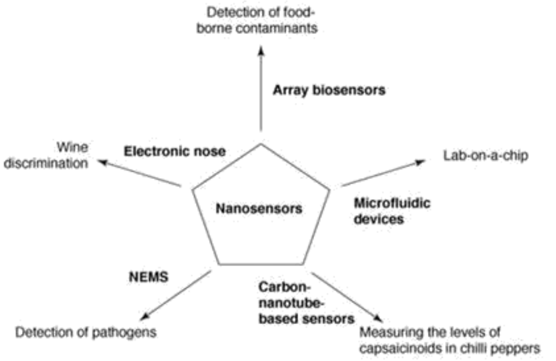

Figure 1. Applications of Nanotechnology in Food Processing and Analysis. *From* Sozer and Kokini, 2009.

Nanomaterials provide conductive or semiconductive features for recognition or catalytic functions facilitating electrochemical or optical detection. For organophosphate pesticides (*e.g.* carbofuran) inhibition of acetylcholinesterase immobilized onto magnetic beads provides detection at the nanomolar level. Lateral flow dipstick based tests for aflatoxin employing gold magnetic nanoparticles with immobilized anti-aflatoxin antibody are available. Similar assays for other mycotoxins including the tricothecenes as well as some agricultural chemicals are under development.

Biosensors for food borne pathogens use copper, silver or gold nanoparticles or quantum dots to enhance optical response (*e.g.* surface enhanced Raman spectroscopy (SERs)). Capture assays for *E. coli* have been developed using glycosylated nanoparticles or bioconjugated silica nanoparticles with monospecific polyclonal antibodies. Several types of enzyme based assays for *Salmonella* spp. (flagellar proteins), *E. coli*, *Listeria monocytogenes* and hepatitis among other disease causing pathogens have been developed; recovery of the pathogen from the food system and associated separations technology remain the greatest impediment for widespread implementation of these tests. Detection limits for foodborne pathogens range from <10- 100 CFU/ml) using biosensor technologies. One of the most unique assays for *E. coli,* (to 50 CFU) uses a screen printed carbon electrode immunosensor with gold nanoparticles and horseradish peroxidase to amplify amperometric response. Nanoproteomic gold based biobarcodes are under development for protein targets in serum and food related applications of this technology should be available soon.

Conclusion

A wide array of biosensors and nanoscale analytical systems employing enzymes as a component for either capture or detection systems have been developed, however many technical challenges remain for simple, rapid and inexpensive detection systems for food components, and chemical or biological contaminants.

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