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Chapter 2

Production, Properties and Applications of Materials-based Nano-Enzymes

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Abstract

Fungi and bacteria cause foodborne diseases and affect food security, which remains the main challenge of the global food industry. Nanomaterials-based enzyme (NMB) technologies play an important role in improving food security issues. This is possible since they can act quickly and efficiently on food substrates when used as biosensors to monitor and control the quality and shelf life of food. This chapter deals primarily with the applications of NMB in the food industry. The production, properties, and applications of nano-enzymes of carbon, zinc oxide, magnetite, copper, and some noble metals in the food industry were discussed. It was suggested that the material could mimic catalytic activities and compete with other naturally occurring enzymes, such as hydrolase and oxidoreductase in foods. It is hoped that this chapter will provide key insights into NMB technologies applied in the food industry.

Keywords

Fungi and Bacteria, Food Safety, Nano-Enzymes Technology, Biosensor, Food Industry

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1. Introduction

The NMB technologies are commonly used as biosensors to monitor and control the physical, biological, and chemical activities of food products [1,2]. They may be considered synthetic materials from metals and non-metals, reduced at the nanoscale (1 - 100 nm) [3,4]. Due to their ability to act as a catalyst to accelerate biological and chemical reactions in food products, they are typically used as biosensors for food application [5]. In the past decades, studies have shown that the catalytic behavior of natural enzymes, such as peroxidase and catalase, are mimicked by many biological materials [6,7]. Many novel artificial enzymes have been developed [6,8–11], and when compared with natural enzymes they are more stable, efficient, sensitive and can be easily modified and recycled [6,12]. The materials can also be stored for longer period thereby enhancing their availability for numerous applications [13]. Typically, the NMB are applied in the food industry for studying and controlling the progress of food products deterioration at molecular scale [12,14–18]. The ability of the material to withstand diverse conditions of pressure, pH and temperature makes them suitable for application in monitoring food quality degradation [13,18].

The catalytic behavior of the material, which is responsible for its unique biosensing properties, can be enhanced by improving its surface area or varying the particles size or composition [19]. Food pathogens like fungi and bacteria can be detected, through application of the NMB, using catalytic colorimetric reaction [7,20,21]. The ability of the

material to detect the early stage of food product defects is especially the reason they are important in monitoring the yield and market value of crop [22]. However, the design of biosensors made them to act rapidly, reliably, and sensitive to environmental conditions [23]. Although, natural enzymes such as catalase and peroxidase can detect food-borne pathogens, the slow reaction and cost has limited their use [14,23]. There are other proven studies reported on the applications of cost-effective NMB in food processing [4,22,24–26].

The current chapter present the application of the NMB, either in combination with other materials or singly, in the food industry. The in-depth knowledge of production and properties of the material, in relation to their actions against bacteria and fungi in food processing were presented. The applications of nano enzymes of carbon, magnetite, zinc, copper, and other noble metals on food products were discussed, and challenges and prospects were also highlighted.

2. Production and properties of nanomaterial-based enzymes

The production or synthesis of the NMB are generally carried out by reducing the particle to nano scale size. This often leads to the production of materials that have some unique properties, such as high surface area, low density, and high solubility of solvents at the nanoscale. Significant progress has been made to design new or improve techniques and approaches for the synthesis of the NMB to meet specific industrial applications. The approach used in the production process may be directly related to how they behave in food applications [18]. On this basis, their production can be categorized as physical, chemical, or biological approaches, as described in the Fig. 1 [27–30].

2.1 Chemical synthesis of nanomaterial-based enzymes

The chemical synthesis of the NMB involves the reduction of ions followed by nucleation, by the reaction of the metal with a reducing agent, in the lattice site of the metal [5,31]. The chemical synthesis of the NMB and structures has been described by Shivashankar [32] in his work on nanomaterial synthesis for different applications. The author uses the chemical vapor deposition method and equilibrium thermodynamics to arrive at a nanocomposite coating with interesting features for industrial applications. The chemical method of synthesis of NMB with excellent active properties was reported by Gaspera [33] in her work on wet chemical synthesis of functional nanomaterials. High-quality synthetic enzymes can also be synthesized using a two-phase approach, which entails a significant reduction in the use of chemicals [34]. The enzymes obtained by this approach usually favor catalytic chemical reaction [35], which is essentially needed in the manufacture of biosensors in the food industry.

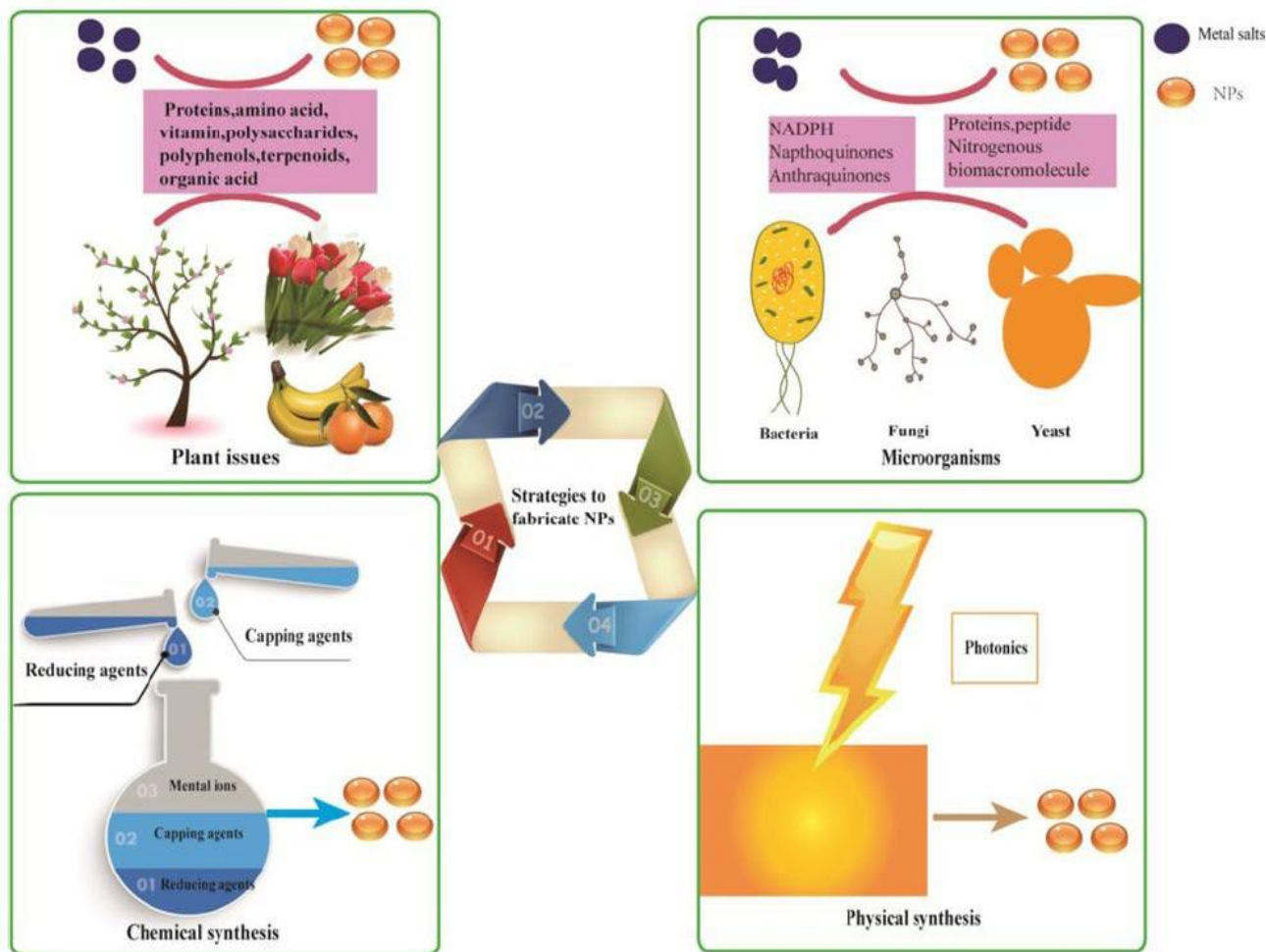


Figure 1: Methods of synthesis of nanomaterials-based enzymes [98]

2.2 Physical synthesis of nanomaterial-based enzymes

The physical synthesis of NMB does not involve the use of chemicals or any biological agent. Several approaches have been reported for enzyme syntheses, laser ablation and condensation methods are common among biosensor manufacturers [19,36]. In the condensation method, the substrate of nanomaterials is normally activated when exposed to temperatures that could render it ionizing and form complex ions that behave like natural enzymes. The method primarily requires adequate space for the combustion activity and a longer duration to complete the pyrolysis or to reach a stable operating temperature [37]. The laser ablation method involves the use of pulse laser irradiation to focus a solid-liquid interface and generate a plasma in the form atoms and ions, which releases quantum of energy in the process [29,38]. This method was used to prepare the high-quality pure NMB [38]. The materials are often associated with a wider distribution of size characteristics that

can slow down its catalytic response capacity, thereby retarding its biosensing application. The problem can be resolved by adding a stabilizing agent in the solution to reduce the size of the material and thus improve the surface [38,39]. This approach was applied by Siegel et al. [40] for synthesizing the silver-gold NMB by stabilizing the resulting solution with glycerol.

2.3 Biological synthesis of nanomaterial-based enzymes

Biological synthesis of NMB usually involves the use of living organisms to induce the conversion of materials into synthetic enzymes. Commonly used living organisms include bacteria [41,42], plant [21,39,43] and fungi [44,45], which can act on the main material substrates and reducing it to stable ions [27]. The ions formed are then used to act and destroy the microorganisms that cause spoilage of the food product [27]. In particular, the addition of bacteria into silver nitrate substrates can reduce the silver ion in the cell to nano-silver, which can act as an artificial enzyme in food processing [41,46]. Since fungi can survive extreme conditions of the environment, the NMB synthesized using the fungi has better reactive properties and is more stable than those prepared using the bacteria [47].

Green synthesis is another biological way of producing enzymes based on nanomaterials. [48]. The resulting enzyme will normally have a moderate level of toxicity compared to other biological synthetic methods, as they can incorporate the metabolite into the biological extract to make them biocompatible [48,49]. The plants such as *Curcuma longa* [50], *Euphorbia hirta* [51] and *Cassia auriculata* [21] can convert metallic materials into enzymes, as they can be used as material agents to reduce the main substrate and later stabilize it [27,52,53]. In particular, the *Lagenaria siceraria* and *Malus domestica* plant extracts can be used to stabilize and reduce noble metals, like silver and gold, to form NMB suitable for application in the food processing [19,46,54].

2.4 Properties of nanomaterial-based enzymes

The NMB used to detect defects in food quality analysis is characterized by its high reactive surface, which can cause it to disperse [55] and interfere with food structure [56]. These properties enable the materials to enhance catalytic chemical reaction between itself and the food substrates during processing, thereby revealing the areas of defects in the substrates. The enzymes can adsorb and interact with the chemical or biomolecules due to high adsorption [57]. This therefore presents the unique ability of the materials for use as detection sensors [58]. The examples of sensors utilizing the NMB includes but are not limited to colorimetric, electrochemical, and fluorescent sensors. Other classes of synthetic enzymes that are useful for detection of estradiol in red wine include silver and gold NMB

[59]. This depends to a large extent on the size of the nanoparticles, which can be improved further to meet other industrial applications [58].

3. Application of nanomaterial-based enzymes in the food industry

3.1 Carbon-based nanomaterial enzyme biosensors

The ability of the carbon-based NMB biosensors to control the activities of deteriorating microorganisms in food and water, thereby enhancing the quality of food and water in the supply chain, have been studied [60,61]. For example, Rao et al. [62] reviewed the numerous potentials of carbon nanotubes, buckminsterfullerene, activated carbon, and graphene oxide for application in food preservation, water purification and other applications. Also, Abdelmonem [63] reported the use of the carbon-based NMB to monitor and control how thermoplastic films can prevent food spoilage and extend its shelf-life. The materials are also used to detect contaminants in liquid foods and to identify or separate spoiled products from many industrial foods [64]. The nanomaterials can in fact be used to control the activities of certain biological reactions that may affect yield in food production [56,64,65]. The incidence of tobacco mosaic virus infection can be suppressed using the nanocarbon biosensor [66]. The protective role of the nanomaterial was made possible by the physical mobility of the carbon ions that attack the virus cells to prevent its further replication [66], as shown in Fig. 2. The aquatic ecotoxicity of wastewater has also been treated using the nanocarbon based enzymes with a great degree of success [67].

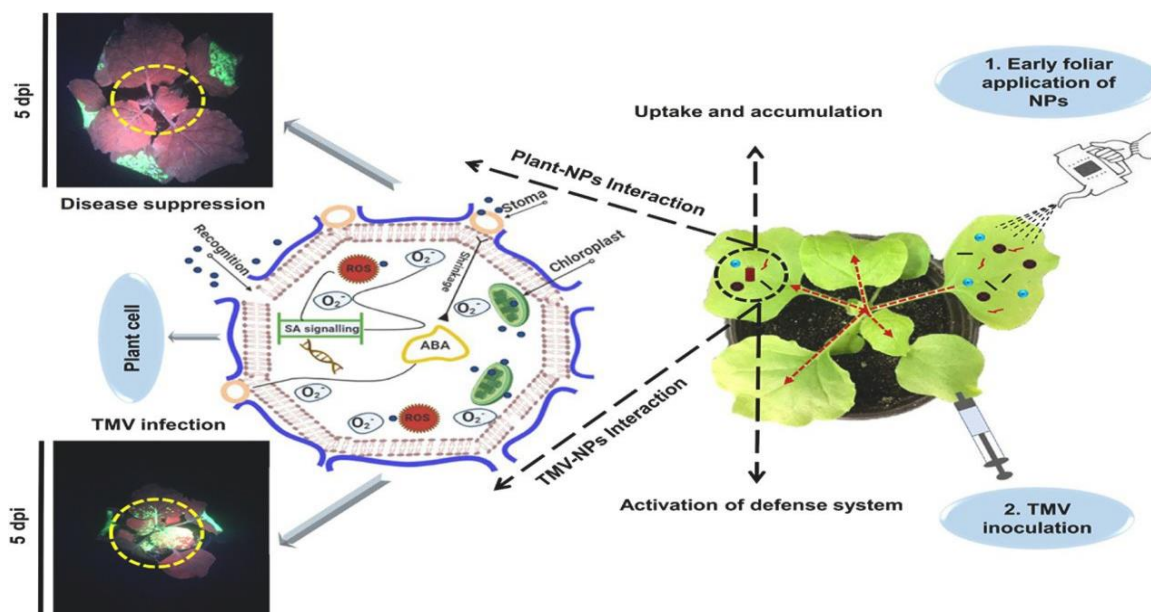


Figure 2: The use of nano carbon-based enzyme to suppress tobacco mosaic virus [66]

3.2 Zinc oxide-based nanomaterial enzyme biosensors

The application of zinc oxide NMB as biosensors to monitor and control postharvest operations in the food sector has been extensively reported in literature [16,68–75]. Akbar and Sadiq [76] applied the zinc-oxide nanomaterials to control the microbial activities in food product. The antimicrobial potential of the zinc-oxide NMB can be associated with the ability of the materials to hydrolyze water to form a collection of hydroxyl ions [77]. This, in turn, will act on the food substrates to target the activities of the fungi and bacterial causing damage to food [25]. Also, the material, when incorporated with acetylcholinesterase, forms an active biosensing product for the detection of pesticides in storage facilities, such as silos, and bans [78]. This biosensor can detect paraoxon pesticide in the range 0.035–1.38 ppm and can be used to detect other acetylcholinesterase inhibiting organophosphate pesticides in stored food products. The material has also been used for detecting and monitoring the extend of adulteration in milk and milk product in the food industry [17]. The detection of analyte in milk using the nano-zinc enzyme biosensor can be visualized in Fig. 3 [17].

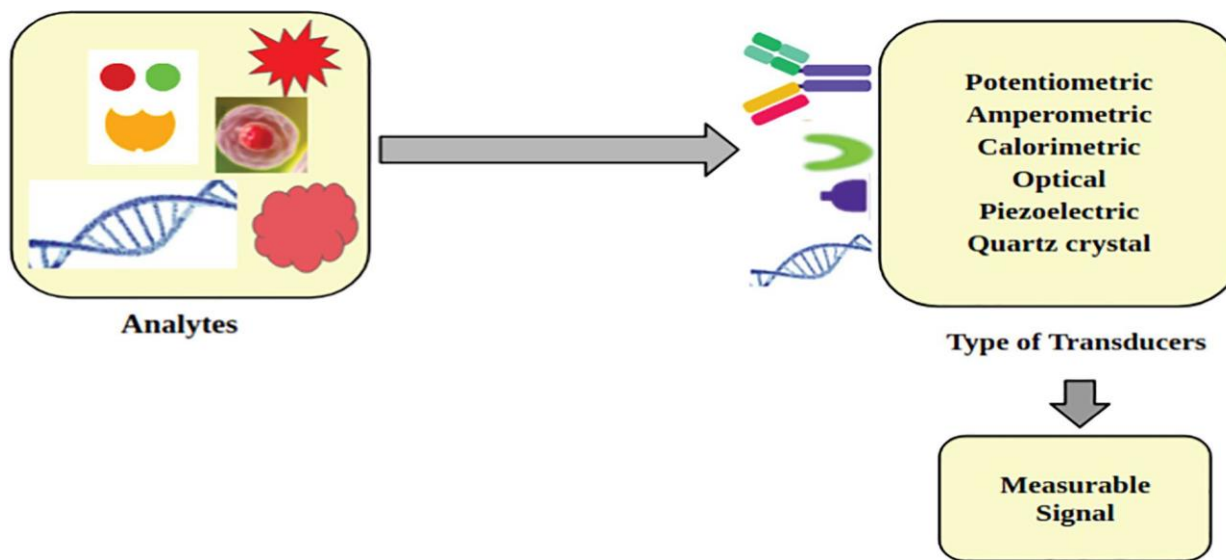


Figure 3: Nano-zinc based enzyme biosensor for detecting analytes in milk [17]

3.3 Magnetite-based nanomaterial enzyme biosensors

The mechanism of magnetite NMB has been explained by Wang and Gunasekaran[15] in their study on artificial enzymes for safety and quality of food. The authors viewed the magnetite nanoparticle as a close replica of the peroxidase natural enzymes with similar properties. We can see how, in Fig 4, the food substrates such as the 3,30,5,50-tetramethylbenzidine (TMB), o-phenylenediamine (OPD), 2,2-azinobis and 10-acetyl-3

(ABTS), and 7-dihydroxyphenoxazine (AR) are oxidized in the presence of the magnetite-based nanomaterials enzymes [15]. This closely imitates the behavior of the oxidase and peroxidase natural enzymes. Other investigators such as Nie et al.[79], Zhang et al. [6] and Gao et al. [8] confirmed the close behaviour of the magnetite NMB with the peroxidase in biosensing applications. Also, the findings of Wan et al. [10] and Wang et al. [11] on the catalytic experiments and steady-state kinetic of the material suggest that the particles undergo enzymatic catalysis just like the natural enzyme peroxidase. The catalytic mechanism was explained by Zhang et al. [9] and Wang [80] to involve the reaction of a collection of hydroxyl ions obtained from the exposure of Fe^{2+} to the hydrogen peroxide. The hydroxyl ion will immediately bind with the hydrogen ion to cause an oxidation of the Fe^{2+} to Fe^{3+} , and the circle repeat itself again. This is how the magnetite NMB achieve its peroxidase like properties, and by this, has often been used as surrogate artificial enzyme in the food industry. The application of the material in food analysis has been extensively studied [15,26,64,81,82]. Other applications of the nano-enzyme have been reported for wastewater and effluents treatment from the food industry [73,83–85].

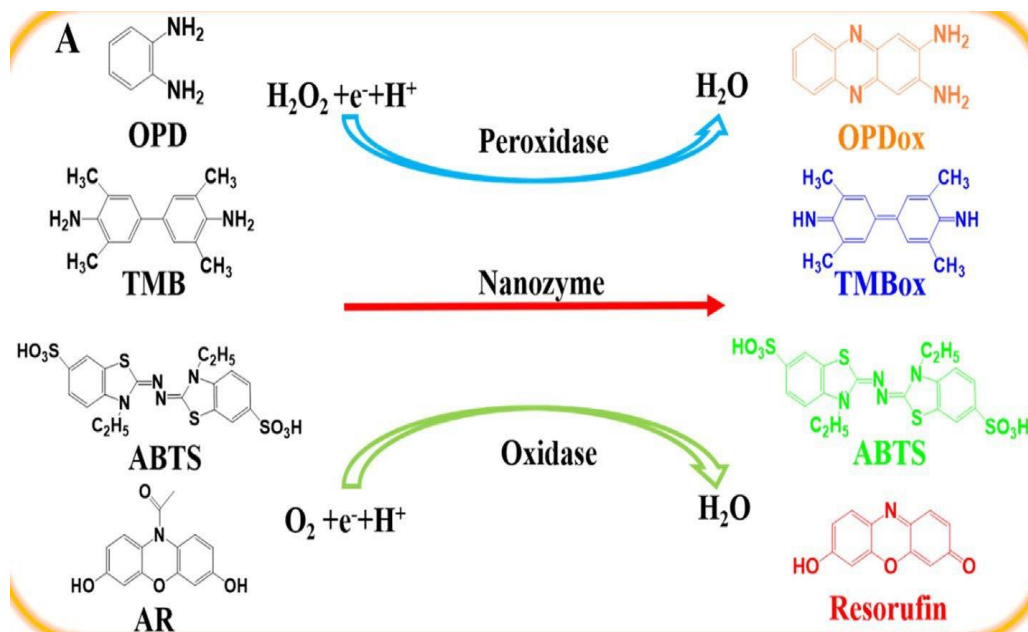


Figure 4: Oxidation reaction of oxidase and peroxidase materials in the presence of magnetite nanoparticles and oxidizing agents [15]

3.4 Copper cluster-based nanomaterial enzyme biosensors

The copper nanocluster NMB exhibits impressive catalytic oxidation reaction between hydrogen peroxide and the substrates. This is due to the ability of copper to exist in two atomic forms in its core, namely five and thirteen, thus indicating different oxidation levels [86]. In the same way as magnetic enzyme biosensors, copper nanoclusters exhibit a higher enzymic behavior than catalase and peroxidase [87]. The reactive mechanism here involves the copper nanoclusters forming bonds with the O-O and the hydrogen peroxide groups to form a substrate and catalytic active sites, which are then rapidly hydrolysed to trigger the oxidation of the substrate. The catalytic response mechanism imitating peroxidase and oxidase enzymes in the oxidative action of the nanomaterial on the food substrate can be seen in the Fig. 5 [7].

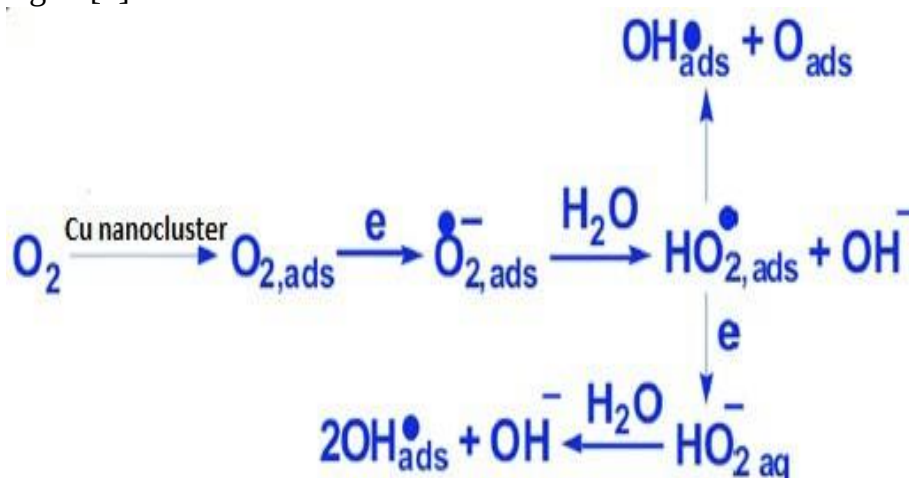


Figure 5: Mechanism of catalytic reaction of copper nanocluster [7]

The copper nanocluster can be used to detect hydrogen peroxide and glucose in food analysis. The action of the NMB here resembles that of peroxidase. The material has been applied in the study of glucose degradation and glucose detection analysis in biomedical and food industries [87]. Other applications of the material are found in the treatment of wastewater and in detecting organic contaminants [2]. Some of the physical properties of the copper-based nanomaterial enzyme which favors its application for wastewater and contaminant treatments include its ability to disperse widely in water, biocompatibility and its high stability when exposed to sunlight [88,89]. Structurally, Bhamore et al. [90] noted that the copper nanocluster has a high affinity to identify or recognize ligand molecules due probably to its transition nature, thus they are suitable for detecting contaminants in wastewater. The detection capability of the NMB can be extended to small ions and internal microstructural defects in food and other applications [2,91–93].

3.5 Noble metal-based nanomaterial enzyme biosensors

The noble metal NMB, which include silver, platinum, and lead ion-based enzyme sensors, can act on food substrates to induce catalytic chemical reactions under wide range of experimental conditions [20]. Although, these materials may show promising abilities in biosensing applications (Fig. 6), they often have a tendency for short shelf-life and may be unstable when expose to extreme conditions. For this reason, they are often used together with stabilizers to help prolong the life span and maintain the stability in food preservation. The combine effect of the materials and the stabilizers on the food substrates has been noted to effectively promote catalytic chemical reaction just like the redox reaction of the natural enzyme peroxidase in fruits and vegetables [94]. For example, the gold nanocluster combined with bovine serum albumin was reported to present high peroxidase-like behavior in wide temperature range and offers improved physical properties than ordinary gold nanocluster biosensors in food processing [12,95].

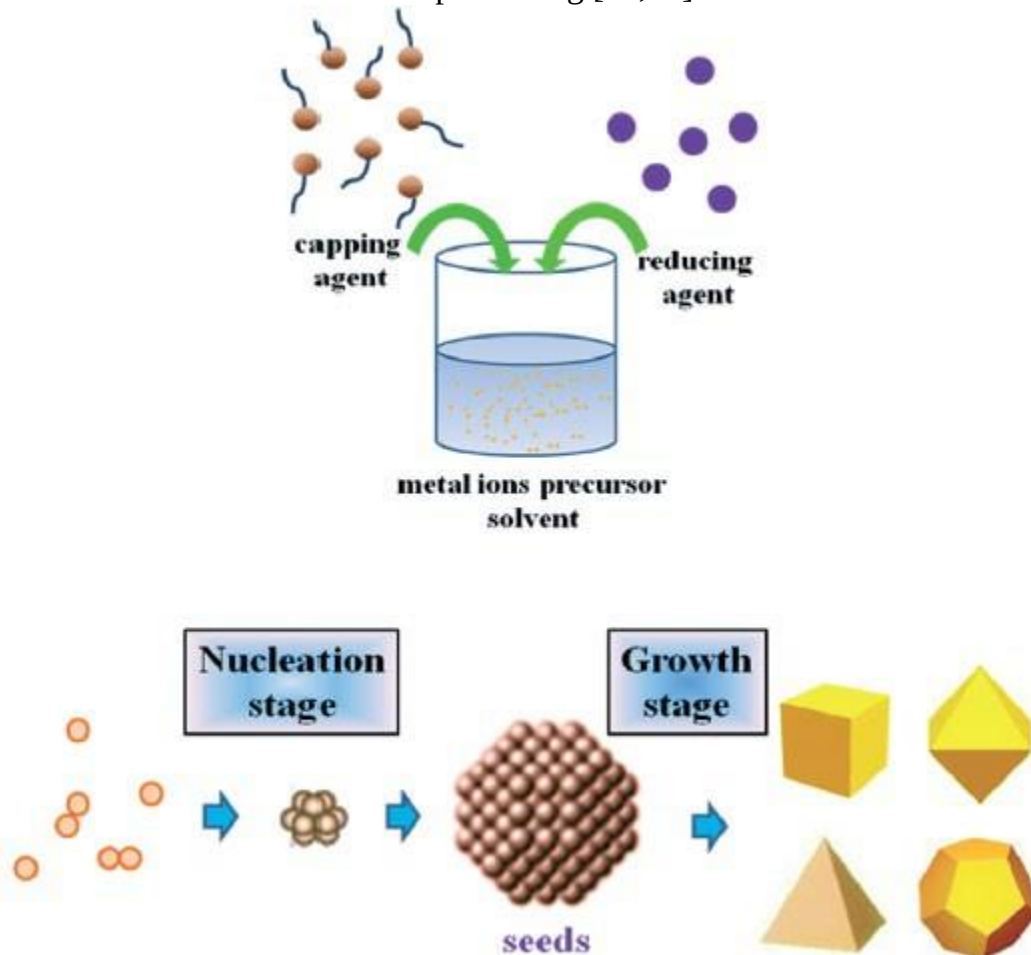


Figure 6: Illustration of synthesis of noble metals nano-enzymes [20]

The application of the noble metal NMB for oxygen scavenging in the modified atmosphere packaging materials maybe possible by hydro-deoxygenation reaction [96]. The material can introduce hydroxyl ions in the storage atmosphere that will inturn react with the excess oxygen to create suitable environment for keeping the food [16,72]. The trimetallic catalytic reaction of the noble metals might be responsible for their unique ability as sensing materials for oxygen. This reaction can also be extended to other types of packaging designs that influence the gaseous composition of the storage atmosphere [14,97]. Also, the noble metals nano-enzymes can be used for detecting incidence of food spoilage due to pest, rodents, or insect attacks [98].

4. Challenges and prospects

After extensive literature review, we noticed that the utilization of NMB in the food industry is currently limited to closely imitating the behavior of natural enzymes, such as oxidase and peroxidase. These two enzymes are the driving forces in the catalytic chemical reactions and changes occurring in food processing and preservation. The natural enzymes are however affected by pH and temperature conditions, which may either speedup or reduce their action on the food substrates. Meanwhile, the nanomaterials are designed to address this deficit of the natural enzymes by providing enzyme like artificial NMBs that can withstand extreme environmental conditions, like pH and temperature. Further research should focus on designing NMB that can mimic the catalytic activities and compete with other natural enzymes including hydrolases, oxidoreductase, transferases, isomerases, and ligases present in foods.

Conclusions

The use of biosensor for the detection of various food pathogens can be traced back to decades, and many of them that are being used nowadays have come from NMB. Because of unique inherent properties, the NMB enzymes have continued to be one of the major bio-sensing technologies still today. In the recent years, there has been a remarkable resurgence of interests observed in the application of the materials in the food industry. With significance usage in monitoring and control of food pathogens, the NMB enzymes have gain new momentum. They are now suitable for effective screening of pathogens in foods. Interaction of the materials with chemical or biomolecules, due to the high adsorption, is one of their main properties, especially in relation to actions against food-borne pathogens. Methods of production and properties of biosensors from various NMB enzymes are available in the literature; the advances and sensitivity of the materials have made their interaction less laborious and easy to apply. The current chapter presents the application of NMB enzymes in the food industry.

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