https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 4 (2025); 36 - 50
e-ISSN:3092-8214 p-ISSN:3092-8206
doi.org/10.33003/chemclass-2025-0904/04

# Recent Advances and Future Trends in the Application of Nanosensors for Food Allergen Detection

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**Abstract**

The increasing incidences of food allergies have become an issue of serious concerns in food safety and global health. Sequel to this, various conventional methods such as Polymerase Chain Reaction (PCR), Enzyme Linked Immunosorbent Assays (ELISA), Western Blotting Method (WBM) amongst others were put forward for the detection of food allergens. These methods however have their inherent limitations. The need for innovative approaches to allergen detection in food therefore becomespertinent. Sequel to this, the emergence of nanosensors has become promising owing to their numerous merits over the traditional approaches to allergen detection. Nanosensors make use of the outstanding physicochemical properties of nanostructure materials. With the advanced sensing mechanism employed in these devices, they are capable of simultaneous and real-time detection of multiple targets at very trace concentrations in food matrices. Furthermore, the minimal preparatory requirements of samples aid their applications in different food matrices and enhancing their usage in complex food materials. To this end, this review explores the recent advances in the application of nanosensors for allergen detection in food. It highlights the types and mechanisms of food allergen. It analyses the most recent literature on the detection of food allergens using nanosensors. The various kinds of nanosensors for allergens detection also discussed. As one of its kinds, it further presents the integration of nanosensors and biosensor for allergen detection, emerging technologies for the detection of allergens and future trends in this regard. Recommendation of areas for

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further research were made, which will lead to the improvement of nanosensors in the detection of food allergens.

**Keywords**: Biosensors, Food allergens, Nanomaterials, Nanosensor, On-site detection, Sensitivity.

## 1.0 Introduction

The presence of allergens in food is an issue of serious health concern globally. This is because it affects a remarkable percentage of the global population [1]. Recently, there has been recorded increase in the trend and prevalence of food allergies, with more studies attributing the observed trend to numerous factors such as dietary change, environmental factors, and hereditary as driving force for this [2, 3]. Food allergies are proteins with capability of triggering abnormal feedbacks from the immune system. These proteins are found in different food materials and most commonly available foods such as wheat, shellfish, eggs, tree nuts, and soy. The detection and quantification of these allergens in food has therefore attracted researches recently [4].

Numerous conventional methods such as Polymerase chain reaction (PCR) and Enzyme Linked Immunosorbent Assays (ELISA) have been employed for the detection and monitoring of allergens in food, with these methods having their inherent limitations. The need for a rapid, reliable, and highly sensitive technique therefore becomes paramount [5].

With the advent of nanotechnology, the application of nanosensors for the detection of allergen in food has been considered highly promising due to the high sensitivity, selectivity, accuracy and portability of this technique for on-site and real time monitoring. Nanosensors are highly miniaturized devices that use materials at nanoscale dimension (1 to 100 nm) in the detection of chemical and biological substances with high sensitivity and selectivity. The outstanding chemical and physical properties of nanomaterials such as improved reactivity, large surface area, amenability to surface modification, and remarkable optical and electrical properties make them suitable candidates for numerous sensing utilizations[6,7].

The use of nanosensors is therefore considered as a remarkable substitution for the existing traditional methods for allergen detection in food. Sequel to this, various researches are currently on going, on the potential integration of nanosensors with various other sensing techniques for more improved performance in allergen detection in food products. Some of the nanomaterials that have been explored for this purpose include carbon nanotube, quantum dots, and metallic nanoparticles [8].

This review therefore focuses on the recent advances and future trends in the application of nanosensors in the detection of allergens in food. It gives a general overview on allergens in food, their types and mechanism of action. It explores some of the existing traditional techniques for allergen detection with their inherent limitations. Furthermore, it delves into the concept of nanosensors, their mechanism of allergen detection and their remarkable advantages over the traditional techniques. It also highlights some emerging and novel technologies in nanosensors for allergen detection (Figure 1). Finally it attempts some of the future direction in the application of nanosensors in allergen detection.

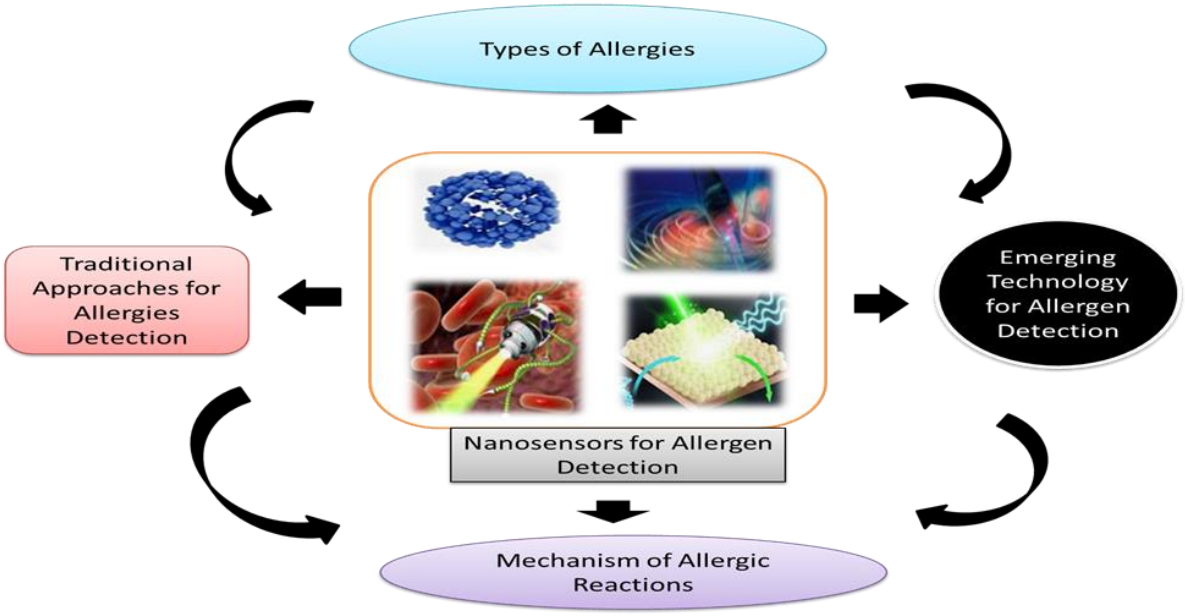



Figure 1: Schematic representation of scope of the review

**2.0 Concept of food allergens and types**

Food allergens refer to special proteins that are found in some food substances that are capable of triggering immune system responses in some people that have sensitivity to them. Such reactions resulting from the trigger are known as allergic reactions (Figure 2). These proteins molecules provoke IgE (immunoglobulin E) assisted reactions on ingestion. The allergic reactions occur because the body immune mechanism identify these proteins to be harmful thereby inducing the production of histamines and related chemicals hence the observed symptoms. Some food allergens also bring about cross-reactivity in which they cause allergic reaction to other similar proteins aside the food itself[9, 10].There are eight major allergens (table 1), commonly described as the 'Big Eight' that

are regulated by the food safety laws in most countries.

**Table 1: Types of allergens, associated proteins and distribution**

| Allergens | Associated protein | Age distribution | Populations/region affected | Reference |
|---|---|---|---|---|
| Eggs | Ovalbumin and ovomucoid | Children and infants especially those with atopic conditions | Globally spread | *[11]* |
| Celery | Api g 1 and 2 | Children and adults | Europe | [12] |
| Tree nuts | Pru du 6 in almond and jug r 1 in walnut | Common among children | Western populations | [4] |
| Peanuts | Ara h 1, 2 and 3. | Childhood | Western countries | [8] |
| Sesame | Sesamin and sesamolin | All ages | Globally spread | [13] |
| Shellfish | Tropomyosin | All ages, most especially childhood | Global spread | *[14]* |
| Milk | Whey protein and casein | Children and infants | Lactose intolerant adults across different countries | [15] |
| Fish | Parvalbumin | Children and adults | Coastal regions | [6] |
| Mustard | Sin a 1 and 2 | Children and adults | In regions where mustard is well consumed | *[16]* |
| Soy | Beta-conglycinin and glycinin | Children and infants | Common among children and infants with eczema | [13] |
| Wheat | Gluten and Gliadin | Infants and children | Children population across different regions | [4] |

**3.0 Mechanisms of food allergies**

Allergies involve a response from the immune system during which the body wrongly identifies certain proteins to be harmful. The responses are categorized into two primary types:

i. IgE-mediated allergies: Here, the immunoglobulin antibodies attach to the allergens, thereby inducing the release of chemicals such as histamine from basophils and mast cells. This takes place immediately after exposure.

ii. Non-IgE-Mediated Allergies: These reactions do not show immediately there is exposure and the IgE antibodies are not involved in the process. A typical example is the enterocolitis syndrome, induced by food protein [4].

**4.0 Importance of allergen detection in food**

The occurrence of food allergies in amongst individuals is an issue of serious health concern, since it affects millions of individual globally with complications varying from mild to more severe cases. Based on the statistics from the

Center of Disease Control and Prevention (CDC), an approximate of 4-6% children and 4% adults are susceptible to food allergies, which call for the need for proper detection of allergens very vital [10]. With the rising cases of reactions emerging from exposure to some food there is need for strict measures for ensuring food products are safe because even low amount of allergens can induce the allergic effects. Allergen detection in food is pertinent as it aids in the prevention of exposure of people to allergens mistakenly[17]. Accuracy in the detection of allergen is vital for proper labeling practice in ensuring the safety of consumers. Allergen detection is also critical in ensuring regulatory compliance. Various countries have put forward strict regulations and measures in the labeling and monitoring of different food products [18]. With the rising prevalence of food allergens, the quest for sensitive, advanced and novel detection techniques has become pressing with a view to building the confidence of consumers, ensuring human health safety and meeting standards sets by regulatory agencies[16].

## 5.0 Traditional methods for the detection of allergens in food

There are various conventional approaches for the detection of allergens in food (Figure 2). Each of these approaches has its merits and demerits. Basically, the choice of a particular traditional method is dependent on certain factors such as nature and type of the food matrix, the particular allergen, and the specificity and sensitivity required[19]. Some of the traditional methods are discussed as follows.

### 5.1. Visual Inspection Method

This method is used in various industries, healthcare and pharmaceuticals for the identification of allergens. It involves the careful inspection of the ingredients, products and environment.This method has certain advantages such ease of implementation, saves time and it is very cost effective. It however has some limitations such as poor sensitivity, possibility of cross contamination, and it high subjectivity. Hence commonly used as a complementary approach.

### 5.2. Enzyme-linked ImmunosorbentAssay (ELISA)

This is one of the most commonly employed techniques for detection of allergen. It uses an antibody that is enzyme linked in the detection and quantification of allergen. It is cost effective, fast and capable of detecting multiple allergens the same time. It is however limited in that it requires a known allergen or antigen for coating, easily affected by matrix effect, and not effective in detecting allergen at trace concentration[20].

### 5.3. Western Blotting Method (WBM)

It combines immunodetection with gel electrophoresis in the detection of allergen. It is

an efficient method for studying the modification and interaction of allergens in various matrices. It is useful as a confirmatory test for other methods such as ELISA. This technique is however highly time consuming, less sensitive and requires in-depth knowledge of proteins biochemistry in the selection of antibody.

5.4. Dot Blot Assays

This is a simplified version of immunoassay method for the detection of allergen in the surface of a solid. It involves the dotting of the sample containing the protein on a membrane and then the membrane is dried, incubated with a blocking solution and subjected to incubation before analysis of the result. It is a simple and rapid technique. It is however suitable for qualitative test and less accurate for the quantitative determination of allergen.

5.5. Polymerase Chain Reaction

This technique focuses on the genetic material of the allergen unlike other methods such as ELISA that depend on the protein detection. It amplifies the characteristic DNA sequencing enhancing detection even at trace level. It is however limited because of its yield of false positive results occasionally and requires highly specialized training.

5.6. MatrixAssisted Laser Desorption/Ionization Mass Spectrometry

This is an analytical technique that has been well employed for allergen detection. It combines ionization with laser desorption. It requires a minimal preparation of sample and hasthe potential of analyzing complex matrix without need for extensive separation.It however has some limitations in allergen detection which include vulnerability to matrix effects which can result to inconsistent results and the need for careful validation and calibration.

5.7. HighPerformance Liquid Chromatography (HPLC)

This is a versatile technique for allergen detection in food. It has the potential of differentiating between similar allergens and proteins and provides quantitative data on the contents of the allergen in food which is vital for regulatory compliance and labeling. It however requires a tedious step of sample preparation which is time consuming and therefore not suitable for rapid detection of allergen which is one of the major quest in food industries. The HPLC system is also expensive, large and difficult to maintain.

5.8. Gas chromatography

This is employed in detection of allergens in certain food products and other samples from the environment. Though GC is most commonly used in the analysis of very small molecules, it can be employed for allergen detection when there is a special sample preparation step. The development of robust GC methods for the detection of some specific allergens is challenging and cumbersome.

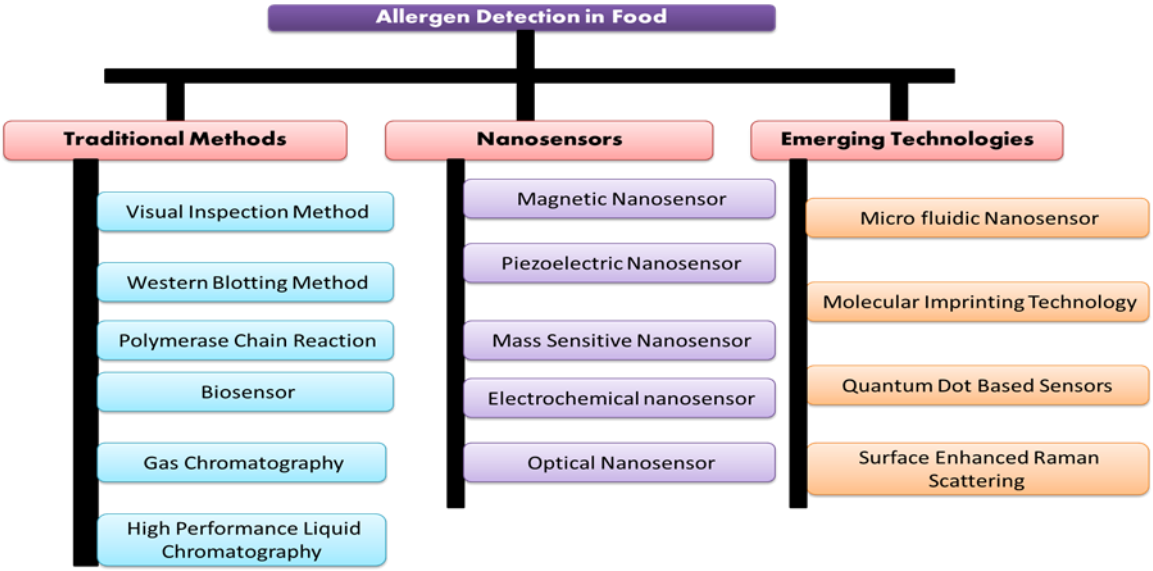



Figure 2: Technologies for the detection of allergens in food

**6.0 Application of nanosensors in allergen detection**

There are various advantages associated with the use of nanosensors in the detection of food allergens (Table 2). Their high sensitivity aids the detection of allergens even at very trace concentration which is very paramount in the protection of individuals that are very sensitive to allergens. Conventional techniques such as ELISA may find it difficult to detect allergens at extremely low concentrations. Nanosensors are also known for their rapidity in the detection of allergens, which is a vital criterion in the adoption of analytical technique for real time and on site applications where decision must be reach quickly [21]. Also the miniaturization of nanosensor makes them highly portable for various environmental usages. In addition, the incorporation of IoT (Internet of Things) with nanosensors paves way for a brighter future in food safety. By introducing nanosensors during food packaging process, it becomes easier to detect and track different allergens during transportation and storage making it possible for faster decision on safety.

**Table 2: A comparison of nanosensor technology and conventional methods for allergen detection in food**

| Criteria | Traditional method | Nanosensor technology | References |
|---|---|---|---|
| Scalability | Restricted scalability and needs specific laboratory protocols and set ups. | Higher scalability and easily integrated into various point-of-care systems. | [22] |
| Speed of detection | Takes longer time for processing (order of hours and days) | Speedy detection of allergens (minutes) | [23] |
| Cost | Initial cost could be lower but the equipment needed are very expensive | The initial cost may be higher but very cheap to maintain after design | [22] |
| Sensitivity | Relatively lower sensitivity, difficulty in detection trace concentrations | Remarkably higher sensitivity with ability to detect very low concentrations | [20] |
| Portability | Large equipment and usually positioned | Highly miniaturized and portable | [22] |
| Specificity | Moderate specificity and prone to cross reactivity | High specificity, making it possible for tailoring towards targeted allergens | [20] |
| User friendliness | Complex to handle, requiring technicians | Very user friendly | *[15]* |
| Environmental friendliness | A wet process involving use of chemical reagents | A green approach and friendly to environment | [23] |
| Preparation of sample | Requires time consuming and complex stages | Highly simplified preparation | *[21]* |

## 7.0 Types of nanosensors for allergen detection and their mechanisms

There are various types of nanosensors used for the detection of allergens in food. They also have varying mechanisms of detection.

7.1. Magnetic nanosensors

Magnetic nanosensors use magnetic nanomaterials that can be adapted to detect specific allergens. The detection process involves a change in the magnetic field when the allergens bind. This alteration in the field can be detected through different resonance techniques. A variation in magnetic relaxation makes it possible to quantify the allergen [21].

7.2.Field-Effect Transistor Based nanosensor

The functioning of this nanosensor is through the detection of variations in electrical currents induced by allergen binding. On interaction with

the sensing layer, the allergen modulates the concentration of the charge carrier within the substrate, giving rise to quantifiable signals in the source current. This sensor makes it possible for on-site measurements due to their remarkable miniaturization [24].

7.3. Piezoelectric nanosensor

This uses the potential of some materials in generating electric field in response to mechanical stress. The allergen proteins bind to the surface of the nanosensor, causing an alteration in the mechanical properties, such as elasticity or mass, resulting in change in the amplitude or frequency of the vibrations. The high selectivity and sensitivity of this nanosensor has been attributed to nano range dimension which is responsible for the amplification of the mechanical network between the surface of the sensor and the allergens [21].

7.4. Mass sensitive nanosensors

Common examples of this sensor are the quartz crystal microbalance (QCM) sensors. It measures small changes in the mass of the surface of the sensor resulting from the binding of the allergen. There is increase in mass when the allergen binds on the surface of the sensor this brings about a measurable frequency in the quartz crystal, which reflects the concentration of the allergen. The sensor's sensitivity can be enhanced through the functionalization of the surface of the crystal using aptamers or specific antibodies that recognizes the allergen of interest [18].

7.5. Electrochemical nanosensors

This is one of the most common sensors for detection of allergens. This uses electrodes made of nanomaterials for the amplification of the electrochemical signals that are generated from the interaction between the sensor and the allergenic protein. A redox process occurs when the allergen binds to the surface of the sensor, which gives rise to a measurable current. The conductivity is increased using gold nanoparticle or carbon nanotube [19].

7.6. Optical nanosensors

Their mechanism of functioning is based on the properties of light as they interact with the allergenic molecules. One of the foremost principles in optical nanosensor is Raman spectroscopy. It detects vibrations within molecules thereby providing information on the specific compositions of the allergens. The nanomaterial large surface area increases the Raman signal remarkably thereby allowing the identification of the protein in complex matrix. Another mechanism involved in optical nanosensor is the surface plasmon resonance (SPR), which is based on free electron oscillation at the dielectric interface of the nanomaterial. When the allergen molecules bind to the surface of the nanosensor, there is a shift in signal which can be measured quantitatively.

**8.0 Emerging and novel technologies in nanosensing for allergens detection**

There are numerous emerging technologies that integrate nanosensors in the detection of food allergens. Some of these technologies are discussed and also presented in table 3 below.

8.1. Surface Enhanced Raman Scattering (SERS)

This is an advanced technique that utilizes the electromagnetic improvement of Raman scattering by metallic nanomaterial. This makes it possible for the detection of allergens of very low concentrations and abundance. The detections of numerous kinds of proteins like peanut allergens at remarkably high specificity and sensitivity has been made possible using the SERS- based sensing device. More currently, other techniques involving the integration of aptamers with SERS have emerged with promising results.

8.2. Microfluidic Nanosensors

The formation of a highly automated and miniaturized system has been made possible by the integration of microfluidic sytems with nanosensors. Small amount of samples are easily manipulated and adapted for real time detection of allergens using microfluidic devices. Improved speed and sensitivity have been ensured using this system.

8.3. Molecular Imprinting Technology (MIT)

This enhances adapted identification sites in a polymer for some specified allergens. This technique makes it possible for enhanced selectivity and sensitivity in the nanosensor. The detection of various kinds of allergens at very low concentrations has been made possible by the incorporation of metal nanoparticles into MIT.

8.4. Quantum Dot Based Sensors (QDBS)

The outstanding physicochemical properties of quantum dots, makes them very suitable and adaptable for use in biosensing applications. Multiple and simultaneous detection of allergen in food is made possible using this technology.

8.5. CRISPR-based System

This approach is well known for its gene editing potential. Nanosensors based on CRISPR have been developed by researchers for the detection of various kinds of protein sequences associated with the allergen. This approach therefore makes provision for improved sensitivity and selectivity for allergen detection.

**Table 3: Novel technologies that integrate nanosensors in detection of food allergens**

| Novel technology | Unique features | Merits over existing technique | Potential challenges | References |
|---|---|---|---|---|
| Molecular imprinting | Highly tailored molds made of polymers with capability of recognizing characteristic allergen shapes | Robust and cost efficient once it has been established | Development of implanted polymers is usually restricted to known allergens and it consumes time | [24] |
| Microfluidic nanosensors | A miniaturized technology that uses small volume of fluids | Requires a reduce quantity of reagent and enhances speedy analysis | There is difficulty in integrating this with the existing technologies | [19] |
| Surface enhanced raman scattering | Aids raman signals through plasmonicnanomaterials | Highly sensitive at low concentration and enhances a detection that is label free | It needs a very careful calibration and the sensitivity is affected by nature of substrate | [5] |
| Quantum dot based sensor | Uses a highly photostable and tunable fluorescence | The strong optical signal makes it highly sensitive with multiplexing potential | It is prone to issues of biostability and and biocompatibility | [25] |
| CRISPR-based system | It is an RNA aided detection that is programmable | Aids real time detection with high precision | It is prone to potential reactions that are not targeted | [24] |
| DNA Aptamer functionalized sensors | Functions based on specific binding potential of DNA aptamers | High useability and specificity | Limited stability of aptamer | [4] |
| Carbon nanotube arrays | A large aspect ratio and electrical conductivity | Improved sensitivity and transduction of signal | Challenge in large scale production | [18] |
| Nanoimmunosensors | Utilization of nanomaterials in the enhancement of immunoassays | Remarkably high sensitivity in comparison to traditional immunoassay | Issues of specificity with respect to bindings that are non specific | [25] |
| Bioluminescent nanosensor | Nanosensors that emit light and operates on bioluminescence | Potential of continuous detection and non invasive | Standardization and regularization for biocompatibility | [6] |

| | | | | |
|---|---|---|---|---|
| Enzyme linked nanosensors | Introduction of enzymes based amplification | High specificity and selectivity | Prone to instability of enzymes and inhibition | [24] |

**9.0 Challenges in the development of efficient nanosensor technologies for the detection of allergens in food**

Allergens detection in food is vital for the purpose of public health and ensuring food safety, especially amongst people that have allergies to food. The nanosensor technologies has proven to be very efficient in the detection of allergies, there are however some challenges associated with nanosensor based research, in the fabrication and utilization of efficient nanosensor solutions:

9.1. Cost efficiency: The fabrication and utilization of nanosensor itself is an expensive project. Thus cost-efficiency must be taken into consideration during the development of the various components of the nanosensor, since it will be utilize by different categories of food industries and users [14].

9.2. Durability and stability to environmental conditions: For safety in the practical usage of nanosensors under varying environmental conditions, their overall stability is paramount. Most nanosensors tend to undergo changes when subjected to some varying environmental conditions of temperature, pressure and humidity, resulting to a lower efficiency[6].

9.3. Incorporation into existing techniques: In attempt to enhance the efficiency of nanosensor, integration into some other novel technique is vital. This integration process is however not easy to execute [26].

9.4. Specificity of technique: Complex food samples containing allergens usually contain closely related proteins that can bring about cross reactivity. This becomes an issue of concern even though nanosensors are known to be of high sensitivity as a result of the nanomaterial potential [4].

**10.0 Recommendations for Future Research**

In spites of the recent advancement in the use of nanosensors for the detection for allergens in food, there is need for further studies with primary emphasis in the following areas: With the advent of AI, data analysis in nanosensor technologies should be integrated with AI and machine learning for enhanced real time applications. This would further buttress predictive analytics in the field of food safety.

Nanosensor based researches for allergen detection should extend towards more complex matrices which usually contain numerous interferences that complicate the detection of allergens. Also, other studies should also include the exploration of the multiplexing potentials of nanosensors in the detection of multiple allergens this will be of immense benefits for people with multiple allergies. Furthermore, studies focused

on the improvement of the stability of nanosensors in varying environmental conditions of humidity, temperature amongst others should also be encouraged.Finally, there should be development of programs and educational outreach with focus on various stakeholders with a target of understanding the values of utilizing nanosensors technologies in the detection of food allergens is also paramount.

## Conclusion

The increase concerns for food allergens highlight the serious need for an analytical technique that is rapid, reliable and accurate so as to ensure the safety of public health. The conventional approaches although valuable, are usually of limited application as a result of tedious sample preparation step, poor sensitivity and time consuming. The emergence of nanosensor has proven to be a transformative technology in this regard. The further integration of nanosensors with biosensing techniques holds is highly promising in the advancement of allergen detection. There is need for continued studies that focus on the optimization in the design and fabrication of nanosensors with it views to reducing cost and enhancing their overall reliability.

## Declaration of Conflicting Interest

There is no conflict of interest in this work

## Acknowledgment

The authors are sincerely grateful to their various institutions for creating a conducing environment for research.

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