

Aloe Vera-Based Nanofibers Assisted with 2, 2-Diphenyl-1-Picrylhydrazyl as Colorimetric Sensor for Reactive Oxygen Species

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Abstract: Responding to heightened consumer awareness and evolving dietary priorities, innovative advancements in food packaging technologies have emerged. This study addresses this by introducing a novel colorimetric sensor utilizing reinforced nanofibers of aloe vera gel with the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) marker. Aloe vera gel serves as the foundational matrix, intricately integrated with DPPH through electrospinning. Checkerboard experiments unveil the antioxidant potential of aloe vera against H₂O₂, exemplifying its distinctive color change in the presence of reactive oxygen species (ROS). The addition of aloe vera and DPPH in a polyvinyl alcohol (PVA) nanofiber matrix creates an innovative biosensor platform, offering promising insights for advancing food quality monitoring technologies. This sensing system, characterized by enzyme-free operation, cost-effectiveness, and simplicity, holds the potential for in-field ROS detection.

Keywords: aloe vera nanofiber; colorimetric sensor; ROS; DPPH.

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1. Introduction

Spoilage and degradation of food products are a pressing concern in the food supply chain. Perishable objects' deterioration may be accelerated by changes in temperature, humidity, and oxygen exposure. Because oxidation alters many items' taste, texture, and nutritional content, they can become unpleasant and even dangerous [1].

Food matrices pose a considerable challenge for analysis due to their intricate and diverse nature [2]. Identifying the nutritional makeup and safety of food products, especially trace amounts of chemical compounds and microbes, is a difficult operation that frequently necessitates using expensive and time-consuming analytical techniques, specialized facilities, and qualified personnel. There will always be a need for innovative analytical technologies because they are able to detect minute concentrations of chemical substances or microorganisms, preferably on-site and at a reasonable cost. Technology such as this can perform non-destructive analyses, avoid the need for sample pretreatment, and enable individuals with little or no scientific training to operate them. Various tools are available to

analyze food safety risks, including immunological assays, conventional microbiological techniques, analytical chemistry techniques, and, in some cases, live animal testing. However, these techniques may be time-consuming, expensive, insensitive, or require substantial scientific expertise [3]. Nanotechnology offers a promising solution to overcome the limitations in sensing science. Using nanomaterials' distinct magnetic, electrical, and catalytic capabilities, quick, sensitive, and economical foodborne pathogen detection techniques have been developed. Pathogen detection can be done quickly, precisely, and affordably by using nanosensors [3].

A group of sensors that are placed in a geometric pattern to gather and process electromagnetic, optical, or auditory signals is known as an array sensor [4]. An optical array sensor is a particular kind of sensor that enables visual detection of the generated signal. The process of colorimetry involves recognizing, contrasting, or measuring the color intensity of a colored substance in order to ascertain the composition of a sample [5]. Various analytes influence color in a chemical reaction, changing it from its initial color to a distinctive color. Colorimetry is used to measure how these interactions affect a variety of analytes [6]. Colorimetry sensor arrays for measuring food products are made up of several sensor materials. The existing approaches for identifying and analyzing secondary compounds in food, encompassing chemical spectroscopy, electrochemistry, chromatography, and electrophoresis, are hampered by inefficiency, complexity, sluggish operation, and limited sensitivity. This renders these techniques unsuitable for rigorous chemical analysis. Many of these techniques necessitate complex experimental sets, time-consuming preparations, and exorbitant costs. In order to provide real-time, on-site detection of secondary chemicals, simple, selective, portable, and extremely sensitive sensors must be designed [7].

Reactive oxygen species (ROS) are a metabolic byproduct that affects human health and whose overly high levels need to be found. These compounds are a collection of metabolic waste products that aid pathogenic and degenerative bodily processes [8]. Apoptosis, oxidative tissue damage, and disturbed cellular balance caused by excessive ROS production can all hasten the onset of a number of diseases, including cancer, atherosclerosis, diabetes, chronic inflammatory diseases, cardiovascular diseases, and Alzheimer's [9]. Food safety and nutritional value can be efficiently controlled by quickly detecting ROS using a rapid yet effective sensor. Packages for food contain specially designed colorimetric sensors. When sufficiently concentrated levels of a particular ROS are present, these sensors begin to change color. Visual devices in the food distribution system are then used to read them, allowing customers and control personnel to identify ROS early on [10,11].

According to a broader definition, an antioxidant is any compound that, when present in small amounts relative to those of an oxidizable substrate, considerably slows down or stops that substrate from oxidizing [12,13]. A free radical known as DPPH (2, 2-diphenyl-1-picrylhydrazyl) has an unpaired electron on one of its nitrogen atoms, making it stable. A colorimetric evaluation of antioxidant capability is based on inhibiting the DPPH radical. The highest absorbance of the stable radical DPPH is between 519 and 595 nanometers, and it has a purple color in a methanolic solution [14,15]. This method's essential idea is that the DPPH radical functions as an H⁺ acceptor from a donor molecule, like an antioxidant. Consequently, DPPH is changed into DPPH₂. The medium is now yellow instead of purple, and the colorimetric sensor communicates its signal by changing the color [16, 7].

Since nanofibers are porous and have nanoscaled dimensions, they have the highest surface-to-volume ratio of any nanoscaled material. The amount of active level accessible to

interact with corruption markers is greatly increased by this functionality [18]. Larger surface areas in colorimetric sensors enable greater indicator molecule or receptor binding and increase the sensor's sensitivity and reaction, even to minute levels of spoiling indications [19]. Nanomaterials offer a great substrate for loading and immobilizing sensor components, including dyes or particular receptors for spoiling indicators, in an efficient manner [20].

One flexible method of creating nanofibers is electrospinning, which has shown great promise in food spoilage detection [21]. This technique makes it possible to produce nanofibers with a huge surface area and perfect morphology, which makes them incredibly well-suited for the smooth integration of sensor elements into their structure. These electrospun nanofibers improve the overall performance of colorimetric nanosensors by providing a strong support matrix for different sensor materials [22]. Their remarkable qualities provide a multitude of locations for marker immobilization, allowing for an incredibly selective and sensitive reaction to markers of food decomposition in samples [23].

Aloe vera gel contains over 70 physiologically active substances renowned for enhancing immunity, reducing inflammation, anti-cancer properties, wound healing, anti-aging effects, and diabetes treatment [24]. Antioxidants found in aloe vera extend food's shelf life and nutritious content. As a result, the cosmetic, pharmaceutical, and dietary sectors use it extensively [25]. Recently, there has been an increase in interest in using aloe vera gels and extracts to electrospun nanofibers [26]. Using a liquid medium containing DPPH and aloe vera gel, this study examines the existence of free radicals in the food industry.

2. Materials and Methods

2.1. Chemicals.

Polyvinyl alcohol (PVA) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were sourced from Sigma Aldrich Chemical Co. (Germany). Ethanol (99%), hydrogen peroxide (H₂O₂, 30%), and ascorbic acid were likewise obtained from the same supplier. Aloe vera leaves were sourced from a local supermarket, providing a natural component for incorporation into our experimental formulations.

2.2. Preparation of solutions for making nanofibers.

Aloe vera leaves from the *Barbadensis miller* species were washed, disinfected with 70% alcohol, and segmented. The extracted gel was homogenized using a Bandelin-ww3200 sonicator, resulting in a pink hue. This meticulous process ensured the preparation of high-quality aloe vera gel for our experiments. To prepare a 10% polyvinyl alcohol (PVA) solution with a final volume of 4 ml, 400 mg of PVA powder was accurately weighed and dissolved in 3100 microliters of aloe vera gel. The mixture underwent magnetic stirring at 60°C for three hours. Simultaneously, a 10 mM solution of 4 mg DPPH in 1 ml methanol was prepared, and 400 microliters of this solution, ensuring a DPPH concentration of 1 mM in the final mixture, was added to the PVA-aloe vera solution. The resulting DPPH/PVA/aloe vera solution was stirred at 1000 rpm on a heater stirrer for 20 minutes, yielding a final volume of 4 ml. This homogeneous solution was subsequently employed for electrospinning experiments.

2.3. Fabrication of nanofibers via electrospinning.

The nanofiber fabrication process involved placing aluminum foil on the drum of a four-nozzle model ESY 20 electrospinning device, which served as the fiber collector. Subsequently, a specific amount of Aloe vera gel solutions were introduced into two syringes and positioned at the designated nozzle locations. The electrospinning device was operated at room temperature, with a feed rate of 0.2 ml/h and a nozzle-to-collector distance of 120 mm while maintaining a voltage range of 15 to 17 kV.

2.4. Atomic force microscopy of Aloe vera nanofibers.

To analyze the surface topography of Aloe Vera gel, a 100-microliter aliquot of filtered gel was diluted with 900 microliters of distilled water, resulting in an initial dilution of 0.1. The resulting solution was thoroughly mixed and vortexed before a 0.0001 dilution was achieved through serial dilution. No evidence of turbidity was observed in any of the prepared solutions. Five microliters of each diluted solution were evenly spread on glass slides and fixed for 24 hours. The prepared slides were then imaged by an Atomic Force Microscope (AFM). The JPK Nano Wizard AFM equipped with an ACTA-10 cantilever from Germany was used for surface topography determination. The AFM tip, ACTA-10 cantilever, was scanned over the surface of the slides under Contact mode settings. The scanning parameters were Gain 150.0 Hz, PGain 0.0048, Setpoint 1.0 V, Sum 1.61 V, Lateral Def 0.05 V, and Status was scanner retracted. The surface topography of fixed nanofibers on slides was studied using a JPK-AFM microscope, specifically the JPK Nano Wizard from Germany. An ACTA-10 cantilever and preset parameters were utilized for imaging, outlined in Tables 1 and 2. The device operated in Contact mode, where the AFM tip scanned the nanofiber surface and detected deflection through an optical detector.

2.5. Assessing antioxidant capacity of aloe vera-based nanofibers with DPPH.

This assay was conducted to evaluate the antioxidant capacity of aloe vera based on its ability to reduce DPPH radicals, as indicated by a color change. A checkerboard technique was used to determine the optimal concentrations of aloe vera and DPPH. The first stage involved dissolving DPPH in methanol to create a 0.1 mM solution. The protocol involved the labeling of 6 Falcon tubes. Tube A received 12 ml of original aloe vera gel, and the remaining 5 tubes were supplemented with 6 ml of distilled water each. The aloe vera gel solution from tube A (6 ml) was transferred successively from tube B to the final tube (F) with thorough mixing. The solution in the last tube was adjusted to a final volume of 6 ml. The third stage involved labeling 6 more tubes and adding 12 ml of H₂O₂ stock to the first tube (1). The remaining 5 tubes received 6 ml of distilled water, followed by 6 ml of H₂O₂ in the second tube (2), which was then transferred and mixed with the following tubes. We continued this process until the solution reached 6 ml of volume in the last tube (6). The last phase involved organizing and filling 36 falcon tubes with a standardized H₂O₂ and aloe vera gel solution at six distinct strengths, each holding 2 milliliters. Each tube was then filled to a final volume of 4 milliliters after adding an equal volume of DPPH.

2.6. Colorimetric assessment of the aloe vera-based nanofibers with DPPH.

Six falcon tubes were labeled, and 6 mL of aloe vera gel was placed in the first tube (A). 3 mL of distilled water was added to the remaining five tubes. Then, 3 mL of the aloe vera

gel solution was added to the second tube (B), and this process was repeated until the last tube (F) to ensure equal volume among all tubes. Also, a set of six Falcon tubes was labeled, and 6 mL of H₂O₂ stock solution was placed in the first tube (1). Distilled water was gradually added to the remaining tubes, and 3 mL of H₂O₂ was introduced to the second tube (2). This process was repeated until the last tube (6) to maintain consistent volume among all tubes.

2.7. Sensitivity of Aloe vera-based nanofibers with various concentrations of DPPH for detection of H₂O₂

To determine the DPPH concentration, two 1.5 ml microtubes were set to zero on a balance. Each microtube received a precise measurement of 4 mg of DPPH, followed by the addition of 1 ml of methanol to facilitate thorough dissolution. The ensuing step involved generating three dilutions from the 10 mM DPPH concentration in methanol, resulting in concentrations of 0.01 mM, 0.1 mM, and 1 mM. Subsequently, the first microtube (Tube 6) received 1 cc of 30% H₂O₂. The next step was to fill the remaining five microtubes with 500 µl of distilled water, 500 µl of H₂O₂, and so on till the last tube. Careful mixing was done after each addition. Aloe Vera gel was homogenized, and 50 µl was added to all wells. DPPH and H₂O₂ were then added according to the provided table. The microtiter plate was placed in a microtiter reader, and absorbance readings were recorded at 517nm. Also, as the negative control for the above checkerboard, the experiment was performed using double distilled water, replacing H₂O₂.

3. Results and Discussion

3.1. Characterization of DPPH/PVA/AV nanofibers with AFM.

The AFM analysis conducted on the composite revealed the presence of well-prepared nanofibers, indicating the successful electrospinning process and the successful incorporation of aloe vera and DPPH in the polymer matrix. The AFM results provided valuable information regarding the diameters and roughness of the nanofibers, confirming their successful formation (Figure 1).

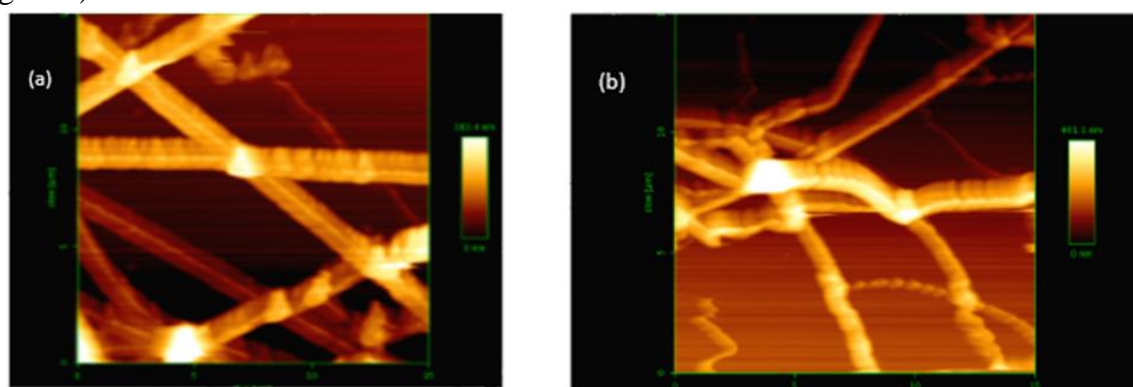


Figure 1. (a) AFM micrograph and roughness indices (Ra 80.79, Rq 104.8, and Rt 739.5 nm) of PVA/AV nanofibers with 1mM DPPH; (b) AFM micrograph and roughness indices (Ra 68.32, Rq 87.14, and Rt 703.4 nm) PVA/AV nanofibers with 10 mM DPPH

3.2. Checkerboard assay for DPPH and aloe vera antioxidant interactions.

The introduction of Aloe vera gel in the assay resulted in a significant interaction with the constant concentration of DPPH, causing a noticeable spread of the purple color and its integration into the gel matrix. This spread varied in different regions of the network, indicating

<https://nanobioletters.com/>

varying levels of antioxidant potential within the gel. Gradual transformations from deep purple to lighter shades and even faint yellow were observed in areas with higher Aloe vera gel content, showcasing the intricate nuances of antioxidant activity within the gel. The addition of H_2O_2 to the assay induced exothermic reactions, providing tangible evidence of oxidative stress neutralization. The intensity of these reactions varied with different concentrations of H_2O_2 , highlighting its ability to counter oxidative stress. Furthermore, aloe vera gel and H_2O_2 co-presence produced complex patterns and colors, demonstrating the multifaceted nature of antioxidant interactions. This fusion of colors created an intricate canvas of potential antioxidant reactions in the system. The observed changes across the network followed a distinct gradient pattern, with stronger responses observed in areas with higher concentrations of Aloe vera gel. This gradient effect (Figure 2) emphasizes the localized and subtle antioxidant properties of aloe vera gel with various concentrations of DPPH.



Figure 2. Visual changes in Aloe vera gel color reacting with various concentrations of H_2O_2

3.2. Color change capability of aloe vera gel in the presence of H_2O_2 .

At the highest level of aloe vera gel, the experiment revealed a significant release of heat when exposed to different doses of H_2O_2 . This reaction became stronger as the H_2O_2 concentrations increased, indicating the potential antioxidant properties of the gel. The color change (Figure 2) from pale pink to brighter shades was consistent and reflected the rise in H_2O_2 concentration. In contrast, the lowest gel concentration showed a weaker exothermic reaction to varying levels of H_2O_2 , suggesting a lower reactivity. Nevertheless, as the H_2O_2 concentration increased, the reaction also intensified, demonstrating the concentration-dependent effects of the gel. The color changes at this level were relatively subtle but still discernible, indicating the subtle influence of lower aloe vera gel concentrations.

3.3. Aloe vera reactivity with various concentrations of DPPH and H₂O₂.

The Aloe vera gel displayed significant changes in color due to the presence of H₂O₂ and DPPH, even when kept at a constant concentration. The initial color of the gel was used as a reference for subsequent colorimetric analyses, which showed noticeable color variations. These changes ranged from a faint transparent pink to a combination of bright pink and subtle yellow, depending on the concentrations of H₂O₂ and DPPH used. This demonstrates the concentration-dependent reaction of aloe vera gel and its potential as an antioxidant (Figure 3).

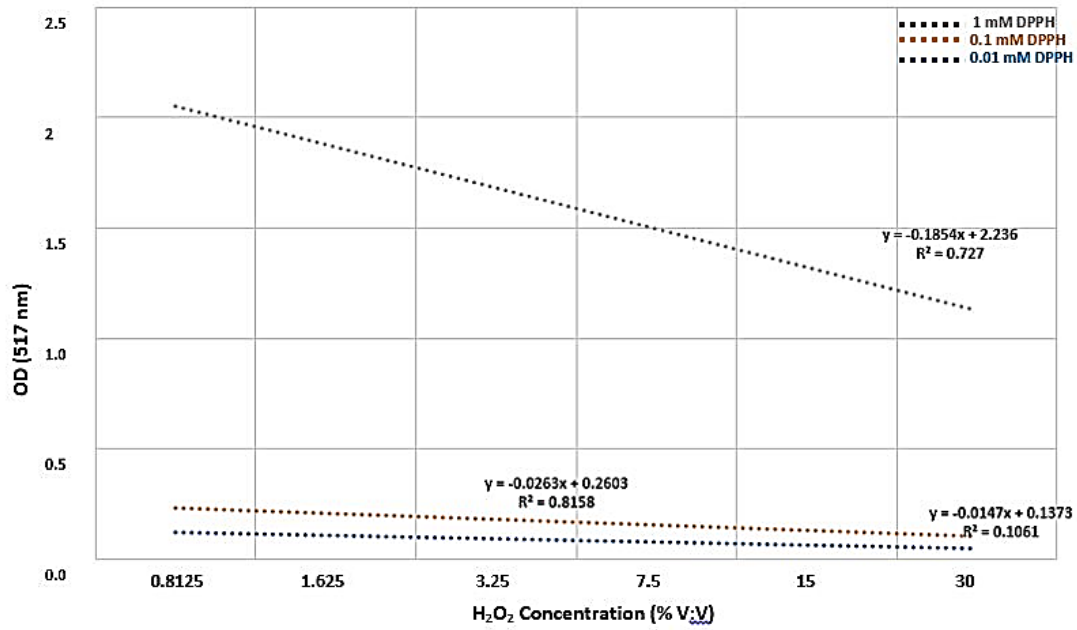


Figure 3. Absorption reading at 517 nm for varied concentrations of DPPH and H₂O₂ in the presence of a constant concentration of aloe vera nanofibers.

Additionally, the absorption measurements were taken at 517 nanometers using a microtiter reader to evaluate the antioxidant interactions between the aloe vera and various concentrations of DPPH and H₂O₂ in a checkerboard pattern experiment (Figure 4).

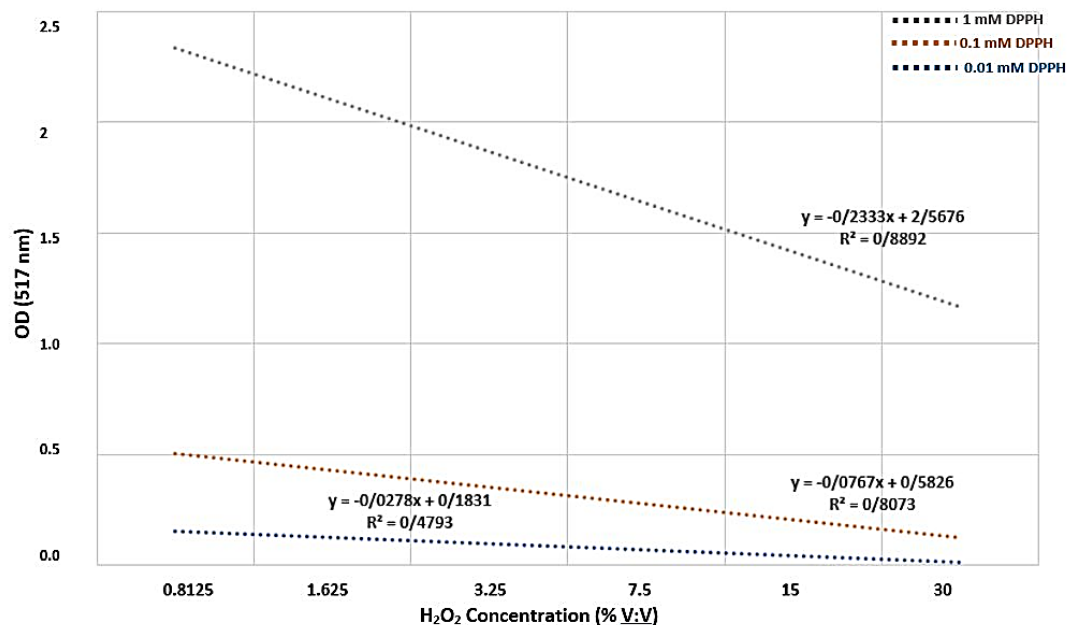


Figure 4. Absorption reading at 517 nm for varied concentrations of DPPH and H₂O₂ with no concentration of aloe vera nanofibers.

In this study, our exploration into novel technical insights has facilitated the visual detection of ROS in food products characterized by diverse natures. Implementing a colorimetric sensor featuring nanofibers derived from Aloe vera gel in conjunction with the DPPH indicator has played a pivotal role in achieving this capability. To scrutinize the topography and roughness of the fabricated nanofibers, atomic force microscopy (AFM) was employed. Capitalizing on the antioxidant attributes inherent in Aloe vera, we extended our investigative approach to encompass checkerboard tests. These tests were meticulously designed to ascertain the optimal concentration of both DPPH and Aloe vera. Through systematic variation of concentrations, our aim was to enhance the synergistic interaction between DPPH and Aloe vera nanofibers, thereby refining the efficacy of the colorimetric sensor for improved ROS detection.

Our study concentrated on assessing the antioxidant potential of aloe vera by observing how the color of DPPH changed when H₂O₂ was present. The design of a checkerboard test that combined Aloe vera with varying concentrations of DPPH and H₂O₂ was a unique feature of our research. This approach aimed to optimize the interaction between Aloe vera nanofibers and DPPH, thereby increasing the sensitivity of the colorimetric sensor for ROS detection. This innovative strategy highlights the importance of considering multiple factors in sensor design. The fabrication of nanofibers from Aloe vera gel using the electrospinning technique was a crucial step in developing the nanosensor. Atomic force microscopy analysis revealed distinct surface characteristics of the nanofibers under different concentrations of DPPH. The nanofibers in 10 mM of DPPH showed Ra, Rq, and Rt values of 80.79 nm, 104.8 nm, and 739.5 nm, respectively, while those in 1 mM of DPPH had values of 68.32 nm, 87.14 nm, and 703.4 nm. These differences emphasized the influence of DPPH concentration on the nanofiber morphology and provided further insights into the behavior of the sensor.

The work conducted by Song *et al.* explores the synthesis and identification of LaMnO_{3+δ} nanofibers for a colorimetric assay targeting the detection of L-cysteine, an amino acid [27]. The nanofibers, fabricated using electrospinning, demonstrated oxidase-like catalytic activity, specifically catalyzing the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) and elucidating the involvement of ROS in this catalytic process. Notably, the study established a colorimetric method reliant on the visual observation of the fading blue color of the catalytic reaction system, indicative of the concentration of L-cysteine. While both studies employ electrospinning techniques for nanofiber fabrication, our work stands apart through the novel application of Aloe vera gel for the colorimetric identification of ROS.

The investigation by Yildiz *et al.* encompasses the preparation of chitosan (CS) and polyethylene oxide (PEO) nanofiber films loaded with curcumin, aiming to monitor freshness in chicken meat through color and spectral changes [28]. The study underscores the potential application of these nanofibrous films as smart food packaging materials responsive to pH variations and ammonia's presence. In contrast, our study introduces a distinct dimension, utilizing Aloe vera gel to design nanosensors specifically tailored for the colorimetric identification of ROS.

The research conducted by Maftoonazad *et al.* involves the electrospinning of nanofibers using polyvinyl alcohol (PVA) and natural pigment extracted from red cabbage for pH biosensing [29]. Their study establishes the effectiveness of the fabricated nanofiber substrate as a pH sensor, showcasing reversible color changes across a range of pH values. While both studies utilize electrospinning techniques for nanofiber fabrication, our work diverges by employing aloe vera gel to design nanosensors explicitly targeted for colorimetric

identification. The focus on ROS detection introduces a unique dimension compared to pH biosensing, showcasing the versatility of nanofiber applications.

In 2020, research was conducted that the study investigated the development of colorimetric sensor films using biodegradable cellulose and naphthoquinone dyes extracted from *Arnebia euchroma* (AENDs) for real-time monitoring of food freshness [30]. The visual observation of color changes from red to purple and bluish-violet indicates food corruption. In contrast, our study introduces a distinctive approach, employing aloe vera gel to fabricate nanosensors specifically designed for the colorimetric identification of ROS in foods. The utilization of Aloe vera gel imparts a novel aspect to our research, contributing to the broader understanding of biosensors and their applications in assessing food quality.

4. Conclusions

This study introduces a pioneering approach in the realm of biosensing, manifesting as the design and fabrication of a nanosensor utilizing aloe vera gel for the colorimetric identification of ROS. The multifaceted methodology employed encompasses preparing Aloe vera gel solutions through freeze-drying and homogenization, evaluating antioxidant capacity through interaction with DPPH and H₂O₂, and the innovative checkerboard test to optimize sensor parameters. The fabrication of nanofibers through electrospinning, coupled with atomic force microscopy characterization, further underscores the meticulous engineering of the sensor.

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Conflicts of Interest

The authors declare no conflict of interest.

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