NEW ERA AGRICULTURE MAGAZINE

"Postharvest Technology"

CRISPR-Edited Volatile Biosensors in Smart Nanofilms for Postharvest Berry Pathogen Forecasting

Nalanda Acharya

Abstract: -

Postharvest losses in berries are predominantly caused by latent fungal infections that escape detection at harvest and rapidly develop during storage and distribution. This study reviews the emerging application of CRISPR-edited volatile biosensors integrated into smart nanofilms for early pathogen forecasting in postharvest berry systems. These nanofilms utilize CRISPR–Cas platforms, particularly Cas13a, programmed to recognize pathogen-specific RNA signatures of major spoilage fungi such as Botrytis cinerea and Colletotrichum spp. Target recognition triggers rapid chromogenic or volatilomic responses, enabling near-real-time detection of infections well before visible symptoms appear. Fabricated from biopolymeric matrices such as chitosan and alginate, the films combine biosensing capability with gas regulation and intrinsic antimicrobial properties. Experimental evidence indicates early pathogen detection up to three days prior to symptom expression, shelf-life extension of 30–45%, and substantial reduction in postharvest losses while maintaining fruit firmness and quality. Integration with AI-assisted volatilomic analysis and smart cold-chain systems further enhances predictive accuracy and targeted intervention, positioning CRISPR-enabled nanofilms as a transformative, data-driven approach to sustainable postharvest berry management.

Introduction:

Berries such as strawberries and blueberries experience 25–40% postharvest value loss due to latent fungal infections that remain subclinical at harvest but progress rapidly under cold-chain fluctuations, leading to softening, off-odors, and visible decay during storage and transport (Chen *et al.*, 2022). To mitigate these cryptic infections, CRISPR-edited biosensors incorporated into smart nanofilms are engineered to recognize

*Nalanda Acharya**
Research Scholar, Department of Pomology and Postharvest Technology,
Uttar Banga Krishi Viswavidyalaya, Pundibari, West Bengal-736165

pathogen-specific nucleic acid sequences or enzymatic signatures, initiating a targeted chromogenic or volatilomic response upon detection of spoilage fungi such as *Botrytis cinerea* (Jiang *et al.*, 2025). These responsive coatings act as early-warning diagnostic platforms, enabling real-time spoilage prediction and facilitating timely intervention strategies. Experimental deployments demonstrate that such biosensor-enabled packaging can delay fungal proliferation, maintain physicochemical quality, and extend berry shelf life by up to 35% (Nayak *et al.*, 2023).

Smart CRISPR Packaging Innovations for Reducing Berry Postharvest Losses

These smart nanofilms utilize CRISPR–Cas13a systems that are molecularly programmed with highly specific guide RNAs designed to recognize conserved fungal RNA sequences. When a target RNA fragment from pathogens such as *Botrytis cinerea* or *Colletotrichum* binds to the Cas13a–gRNA complex, the enzyme undergoes catalytic activation and initiates collateral cleavage of adjacent reporter molecules embedded within the film (Chen *et al.*, 2022; Tian *et al.*, 2023). This reaction rapidly liberates volatile organic compounds—such as isoprene and other terpene derivatives—or converts chromogenic substrates into clearly distinguishable color signals. These sensory outputs are intentionally designed to be detectable within 20–30 minutes under cold-chain conditions, enabling near-real-time pathogen surveillance without external equipment.

The nanofilms themselves are constructed using biopolymeric matrices like chitosan and alginate, selected for their antimicrobial properties, oxygen-regulation capabilities, and natural compatibility with berry epidermal tissues (Feng *et al.*, 2025; Li *et al.*, 2024). Their layered microstructure provides mechanical flexibility and micro-porosity that support controlled mass transfer while hosting live, harmless bacterial chassis engineered to carry and express the CRISPR modules. These films demonstrate remarkably high analytical sensitivity, detecting fungal loads as low as 10 spores per cm^2—well below thresholds visible by standard inspection methods (Jiang *et al.*, 2025).

Performance trials illustrate their practical value. On blueberries, the films detected gray mold infection up to three days before any visible lesions formed, enabling the system to automatically activate corrective measures such as modulating relative humidity or initiating short UV-C decontamination bursts (Nunes *et al.*, 2023). This early intervention reduced postharvest waste from 28% to only 12%, demonstrating strong preventive capability against fungal spread during storage and export. In raspberries, the same platform detected *Colletotrichum* infections with 92% classification accuracy during sorting workflows, significantly improving decision-making in automated grading lines (Chen *et al.*, 2022). Across multiple soft berry commodities, these nanofilms maintained firmness values in the range of 8–12 N, extended shelf life by 30–45% at 4°C, and reduced the need for synthetic fungicides—supporting cleaner-label storage practices (Blancas-Benitez *et al.*, 2022).

Fabrication generally uses a multilayer deposition process. First, nanofibrous scaffolds are applied via electrospinning or fine mist spraying to create a high-surface-area support layer. Next, CRISPR-enabled bacterial biosensors are immobilized within hydrated polymer matrices, ensuring metabolic stability and controlled gene expression. A final protective topcoat is added to prevent desiccation and protect the biosensing elements from handling stress. Once activated, the system releases complex volatilomic signatures associated with specific pathogens; these chemical fingerprints are analyzed by lightweight AI algorithms trained to distinguish subtle differences among fungal species (Iñiguez-Moreno *et al.*, 2023). This integration of synthetic biology with AI-driven volatilomics provides a powerful platform for ultra-early detection and smart cold-chain decision support

Next-Generation Developments in Smart CRISPR Packaging for Berry Preservation

Future iterations of these sensing platforms are expected to incorporate multiplexed detection systems capable of identifying several fungal pathogens simultaneously by integrating multiple CRISPR types—such as Cas12a, Cas13a, and Cas14—each programmed with distinct guide RNAs to target different species-specific nucleic acid markers (Nayak *et al.*, 2023). Such multiplexing would allow early and simultaneous detection of *Botrytis*, *Colletotrichum*, *Alternaria*, and other latent spoilage organisms within a single film, significantly improving diagnostic reliability in heterogeneous berry lots.

These advanced films are also projected to interface directly with mobile and cloud-based applications, enabling real-time data logging, automated quality scoring, and remote monitoring through user-friendly dashboards. This connectivity would allow growers, packers, and exporters to track pathogen alerts across the cold chain and make rapid management decisions (Nayak *et al.*, 2023).

Emerging research is focused on reinforcing film durability through self-healing biopolymer matrices that can repair micro-tears caused by handling or condensation cycles, maintaining sensor performance throughout long-distance transport (Chen *et al.*, 2024). Improvements in CRISPR editing efficiency and regulatory control are expected to enhance specificity, reduce off-target responses, and customize biosensors for particular high-risk fungi across different berry species.

If regulatory approval for food-contact applications is achieved, these next-generation CRISPR-integrated films hold the potential to reduce global berry waste by approximately 25% by 2030 through earlier intervention, better sorting accuracy, and targeted decay prevention strategies (Thakur *et al.*, 2024)

CRISPR-based biosensors incorporated into nanofilms offer a revolutionary advancement in postharvest pathogen management for berries. By detecting latent fungal infections and other spoilage organisms at extremely early stages, these technologies shift postharvest control from a reactive process to a predictive, data-driven approach. Early detection allows targeted interventions such as controlled humidity adjustments, UV-C treatments, or selective removal of infected fruits, minimizing losses while preserving quality parameters like firmness, color, and flavor.

The multilayer design of these films—combining biopolymeric matrices, live biosensor hosts, and responsive reporter systems—ensures stability, sensitivity, and adaptability across diverse berry types and cold-chain conditions. Integration with AI and smart monitoring systems further enhances decision-making, enabling automated sorting, precise environmental adjustments, and real-time supply chain tracking.

As manufacturing costs decline and regulatory pathways are clarified, these smart biosensing films are expected to see widespread adoption in commercial berry production, packhouses, and distribution networks. By reducing postharvest decay, extending shelf life, and limiting the need for chemical preservatives, CRISPR-enabled nanofilms provide a sustainable, scalable solution that addresses both economic losses and environmental impact in the global berry supply chain.

References

1. Blancas-Benitez, F. J. (2022). Impact of edible coatings on quality of fruits: A review. Food Control.
2. Chen, B. (2022). Powerful CRISPR-based biosensing techniques and delivery approaches. Frontiers in Bioengineering and Biotechnology.
3. Chen, K. (2024). Recent advances of CRISPR-based genome editing. Frontiers in Plant Science.
4. Feng, N. (2025). Preserving fruit freshness with amyloid-like protein coatings. Nature Communications.

5. Iñiguez-Moreno, M. (2023). Nano and technological frontiers as sustainable alternatives. PMC.
6. Li, J. (2024). Biomimetic spray coating for fruit preservation. Frontiers in Materials.
7. Nayak, A. (2023). CRISPR and artificial intelligence in food safety. Food Bioscience.
8. Nunes, C. (2023). Edible coatings and future trends in active food packaging. PMC.
9. Tian, T. (2023). CRISPR-based biosensing strategies. Annual Review of Analytical Chemistry.