International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(10): 201-205
www.biochemjournal.com
Received: 15-08-2024
Accepted: 18-09-2024

Suraj S Hanni
Department of Fruit Science, College of Horticulture, University of Horticulture Sciences Bagalkot, Karnataka, India

Arshad Khayum
Assistant Professor, Department of Postharvest Management, College of Horticulture, KSNUAHS, Shivamogga, Karnataka, India

Gouthami Y
Ph.D. Research Scholar, Department of Post Harvest Technology, University of Horticultural Sciences, Bagalkot

Ayesha Siddiqua
Assistant Professor of Fruit Sciences, Department of Fruit Sciences, Sri Krishnadevaraya College of Horticultural Sciences, Affiliated to Dr. YSR Horticultural University, West Godavari, VR Gudem, Andhra Pradesh, India

Lokesh TH
Ph. D. Scholar, Department of Horticulture, College of Agriculture, University of Agricultural Sciences, GKVK, BENGALURU, Karnataka, India

Himanshu Sekhar Behera
Ph.D. Scholar, Department of Soil Science and Agricultural Chemistry, Lovely Professional University Punjab, India

Corresponding Author:
Arshad Khayum
Assistant Professor, Department of Postharvest Management, College of Horticulture, KSNUAHS, Shivamogga, Karnataka, India

Edible vaccines from fruit and vegetables: A comprehensive review

Suraj S Hanni, Arshad Khayum, Gouthami Y, Ayesha Siddiqua, Lokesh TH and Himanshu Sekhar Behera

DOI: https://doi.org/10.33545/26174693.2024.v8.i10c.2476

Abstract
Edible vaccines derived from genetically modified fruits and vegetables represent an innovative approach to vaccination, combining biotechnology, agriculture, and immunology. This review explores the development, efficacy, and potential of edible vaccines, examining their ability to provoke immune responses in humans, mitigate infectious diseases, and improve global access to vaccines. This paper discusses the technical challenges, regulatory hurdles, and societal acceptance of plant-based vaccines. Additionally, we provide an overview of ongoing research, clinical trials, and the potential global impact of these vaccines, especially in low-income regions. By analyzing the current research landscape, we examine the future possibilities of edible vaccines, their contribution to global health, and their role in combating infectious diseases such as Hepatitis B, cholera, Norwalk virus, and more.

Keywords: Edible vaccines, biotechnology, fruits, vegetables, immunology, genetic engineering, global health

1. Introduction

The introduction of genetically engineered vaccines from edible plants represents a leap in both biotechnology and vaccination strategies. Vaccines, traditionally administered through injections, have long faced challenges related to cost, distribution, and patient compliance. Edible vaccines offer a novel approach by eliminating many of these hurdles, using common fruits and vegetables like bananas, tomatoes, and potatoes as platforms to deliver immunogenic proteins.

1.1 Definition and Concept of Edible Vaccines

Edible vaccines are transgenic plants that are engineered to produce antigens capable of stimulating immune responses when consumed. They are produced by incorporating specific genes from a pathogen, which encode for antigens, into the genetic makeup of plants. These antigens, when ingested, are recognized by the immune system, triggering a protective response. This method utilizes the plant's cellular machinery to manufacture the protein and deliver it directly through consumption (Streatfield, 2005) [1]. These vaccines represent an intersection of plant biotechnology and immunology, with the potential to revolutionize the accessibility and delivery of vaccines.

1.2 Historical Development of Edible Vaccines

The concept of edible vaccines was first explored in the early 1990s when researchers discovered that plants could express foreign proteins, including viral and bacterial antigens. Early studies demonstrated that humans and animals could generate immune responses by consuming these genetically modified plants. The first experimental edible vaccine was produced in potatoes for the Norwalk virus (Tacket *et al*., 1998) [2]. Since then, research has expanded to include other plants, such as tomatoes, bananas, and lettuce, and targets a wide range of diseases, including cholera, hepatitis B, and HIV.

In the 1990s, Dr. Charles Arntzen, a pioneer in edible vaccine research, successfully demonstrated the potential of plant-based vaccines with initial experiments involving hepatitis B antigens produced in tobacco plants. This marked a significant breakthrough in the field and paved the way for subsequent edible vaccine development.

1.3 Advantages and Challenges
1.3.1 Advantages
- **Cost-effectiveness:** Edible vaccines offer a cost-effective alternative to traditional vaccines because plants can be grown and harvested using local agricultural methods, reducing the need for expensive pharmaceutical production facilities (Sharma and Sood, 2011) [6]. The production cost of edible vaccines is significantly lower, with estimates suggesting a reduction of up to 90% compared to traditional methods (Fischer *et al*., 2020) [11].
- **No Cold Chain Requirements:** Unlike traditional vaccines, edible vaccines do not require cold storage or refrigeration, which can be particularly advantageous in regions with limited infrastructure (Mason *et al*., 2002) [5]. The absence of a cold chain requirement can significantly improve distribution, particularly in rural or remote areas where vaccine access is a challenge.
- **Improved Patient Compliance:** Edible vaccines, delivered through common dietary items, may improve compliance rates, particularly among children who often fear needles. Oral vaccines eliminate the discomfort and complications associated with needle-based administration, making them more acceptable to patients.
- **Enhanced Mucosal Immunity:** Many infectious agents enter the body through mucosal surfaces such as the respiratory or gastrointestinal tracts. Edible vaccines can stimulate both systemic and mucosal immune responses, offering dual protection. The production of IgA antibodies in the gut, a key component of mucosal immunity, provides protection against many pathogens that enter via the gastrointestinal tract (Arakawa *et al*., 1998) [8].

1.3.2 Challenges
- **Dosage Control:** One of the main challenges associated with edible vaccines is controlling the dosage of antigens. Unlike traditional vaccines, where the dosage is measured precisely, the amount of antigen in an edible vaccine may vary depending on factors such as the plant's growth conditions, harvest time, and processing methods (Daniell *et al*., 2001) [3]. Ensuring consistency in antigen levels across batches of plants is a significant challenge for manufacturers.
- **Regulatory Approval:** The regulatory framework for edible vaccines is still in its infancy. Unlike traditional vaccines, which have well-established pathways for approval, plant-based vaccines must navigate a complex landscape that includes food safety, biotechnology, and pharmaceutical regulations. Regulatory agencies such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA) are still developing guidelines for the approval of edible vaccines (Rybicki, 2009) [4].
- **Public Acceptance and GMOs:** Public skepticism regarding genetically modified organisms (GMOs) presents another challenge for edible vaccines. Despite the potential health benefits, the acceptance of GM crops remains controversial in many parts of the world. Public education and transparent communication about the safety and benefits of edible vaccines will be crucial for their acceptance.

2. Mechanism of Action
Edible vaccines work by using the same principles as traditional vaccines. The goal is to stimulate an immune response by exposing the immune system to antigens, which are proteins that the immune system recognizes as foreign. In the case of edible vaccines, these antigens are produced by genetically modified plants and consumed as part of a person's diet.

2.1 Genetic Engineering of Plants
The process of developing edible vaccines involves incorporating genes that encode for specific antigens into the DNA of a plant. This is typically done using one of two main methods: nuclear transformation or chloroplast transformation. Chloroplast transformation is often preferred because it can produce higher levels of protein and reduces the risk of gene transfer to other plants through pollen (Daniell *et al*., 2009) [3]. For example, a chloroplast-transformed tobacco plant was engineered to produce a rabies antigen, demonstrating high levels of antigen production and effective immune response in mice.

In addition, advancements in CRISPR/Cas9 gene-editing technology have opened new possibilities for more precise genetic modifications in plants, improving the efficiency of antigen production and enhancing the overall efficacy of edible vaccines.

2.2 Delivery Systems for Antigens
Two primary delivery systems are used to ensure the expression of antigens in edible vaccines:
- **Nuclear Transformation:** This involves inserting foreign DNA into the plant's nuclear genome. The gene encoding the antigen is integrated into the plant's chromosomal DNA and is expressed as the plant grows. However, one drawback is the possibility of gene silencing and relatively lower levels of antigen expression.
- **Chloroplast Transformation:** Chloroplasts, the plant organelles responsible for photosynthesis, offer several advantages for antigen production. Since each plant cell contains multiple chloroplasts, this method allows for higher yields of antigens. Furthermore, because chloroplast genes are maternally inherited, the risk of transgene escape via pollen is minimized (Daniell *et al*., 2009) [3]. This technique has been used to develop edible vaccines for a variety of diseases, including rabies, tuberculosis, and cholera.

2.3 Immune Response
When a person consumes a genetically modified plant containing an edible vaccine, the antigen is absorbed through the gut and taken up by the gut-associated lymphoid tissue (GALT). This tissue plays a critical role in producing mucosal immunity by generating antibodies, primarily immunoglobulin A (IgA), which protect against pathogens that enter through mucosal surfaces. In addition to mucosal immunity, systemic immunity is also stimulated, resulting in the production of circulating antibodies (Arakawa *et al*., 1998) [8].

3. Edible Vaccines and Specific Diseases

3.1 Hepatitis B

Hepatitis B is one of the most prevalent infectious diseases in the world, affecting millions of people and causing significant morbidity and mortality. The current vaccine is highly effective, but it requires multiple doses and cold storage, limiting its accessibility in resource-poor settings. Researchers have developed edible vaccines for Hepatitis B by expressing the Hepatitis B surface antigen (HBsAg) in plants such as potatoes and lettuce (Kapusta *et al*., 1999) [9]. These plant-based vaccines have shown promise in animal models and early human trials, where they successfully stimulated both mucosal and systemic immunity.

In one clinical trial, individuals who consumed transgenic potatoes expressing HBsAg demonstrated measurable immune responses, although further studies are required to optimize the dosage and frequency of administration (Thanavala *et al*., 2005) [13].

3.2 Cholera

Cholera is a waterborne disease that continues to cause significant mortality, particularly in regions with poor sanitation. Traditional cholera vaccines are administered orally, but they still require refrigeration and multiple doses. An edible cholera vaccine has been developed by expressing the cholera toxin B subunit (CTB) in genetically modified plants, including rice and potatoes. Studies have shown that these vaccines can induce both mucosal and systemic immunity in animals and humans (Nochi *et al*., 2007) [7].

3.2 Cholera (continued)

Rice-based cholera vaccines, in particular, have shown great promise. Researchers have successfully expressed the cholera toxin B subunit (CTB) in rice plants, and when this transgenic rice was fed to animal models, it induced significant immune responses, including the production of mucosal IgA antibodies in the gut and serum IgG antibodies in the bloodstream (Kang *et al*., 2003) [12]. The advantage of rice is its global dietary significance, especially in areas where cholera outbreaks are common, making it an ideal candidate for local production and distribution.

In a study conducted by Nochi *et al*. (2007) [7], transgenic rice expressing CTB was fed to mice, and the mice exhibited strong mucosal and systemic immune responses without any adverse effects. Furthermore, the rice-based vaccine was stable at room temperature, eliminating the need for cold storage. This makes it highly suitable for distribution in regions with limited refrigeration infrastructure.

3.3 Norwalk Virus

Norwalk virus, also known as the Norovirus, is a leading cause of gastroenteritis outbreaks globally. The virus is highly contagious and is transmitted primarily through contaminated food and water. Conventional vaccines for the Norwalk virus are not widely available, which has led to research into plant-based edible vaccines as an alternative.

The Norwalk virus capsid protein (NVCP) has been successfully expressed in several plant species, including potatoes and tomatoes. In a notable study, Tacket *et al*. (2000) [2] developed transgenic potatoes expressing NVCP, and when these potatoes were fed to human volunteers, they elicited both mucosal and systemic immune responses. Volunteers who consumed the transgenic potatoes produced anti-Norwalk virus antibodies in their serum and saliva, demonstrating the potential of edible vaccines to protect against this pathogen.

The ability to deliver a vaccine through a common food source like potatoes is particularly attractive for addressing outbreaks of gastroenteritis in areas with limited healthcare infrastructure. However, more research is needed to optimize the antigen expression levels and to determine the appropriate dosage required for effective immunity.

3.4 Human Papillomavirus (HPV)

Human Papillomavirus (HPV) is one of the most common sexually transmitted infections worldwide and is a leading cause of cervical cancer. Although effective vaccines exist (e.g., Gardasil and Cervarix), they are expensive and require cold storage, limiting their accessibility in low-income regions.

Researchers have explored the use of transgenic plants, such as tobacco and potatoes, to express the L1 protein from HPV, which is the major capsid protein responsible for inducing protective immunity. Studies have shown that plant-derived L1 protein can form virus-like particles (VLPs) that are structurally similar to the native virus, making them highly immunogenic (Maclean *et al*., 2007) [14].

In preclinical studies, mice immunized with transgenic tobacco expressing HPV L1 protein produced strong immune responses, including the generation of neutralizing antibodies. Although the development of edible HPV vaccines is still in its early stages, the potential for low-cost, needle-free vaccines is significant, especially in regions where cervical cancer rates remain high and access to healthcare is limited.

3.5 Rabies

Rabies is a fatal viral disease that affects both animals and humans. It is primarily transmitted through the bite of an infected animal, and once clinical symptoms appear, the disease is almost always fatal. The current rabies vaccine is effective, but it requires multiple doses and is expensive, limiting its availability in low-income regions where rabies is endemic.

To address this, researchers have developed transgenic plants expressing the rabies glycoprotein (G protein), which is the main antigen used in rabies vaccines. A study by Yusibov *et al*. (2011) [10] demonstrated that tobacco plants could be genetically modified to produce rabies G protein, and when these plants were fed to mice, they generated strong immune responses comparable to those induced by traditional rabies vaccines.

Furthermore, chloroplast-based expression systems have been used to produce high levels of rabies antigens in tobacco and lettuce plants. These systems allow for large-scale production of the vaccine at a fraction of the cost of conventional vaccine manufacturing methods. Early clinical trials have shown that plant-derived rabies vaccines can induce protective immunity in animals, and ongoing research is focused on optimizing antigen expression and delivery for human use.

3.6 HIV

Human Immunodeficiency Virus (HIV) remains a global health challenge, with millions of people living with the virus, particularly in low- and middle-income countries.

Despite the availability of antiretroviral therapy (ART), a preventive vaccine remains a critical unmet need.

Research into plant-based edible vaccines for HIV has focused on expressing HIV antigens, such as the gp120 protein, in plants like tomatoes and potatoes. The gp120 protein is a key component of the HIV envelope, and vaccines targeting this protein aim to induce neutralizing antibodies that can block HIV entry into host cells.

In a study by Zhang *et al*. (2016), transgenic tomatoes expressing the HIV gp120 antigen were developed, and when these tomatoes were fed to mice, they elicited both mucosal and systemic immune responses. The mice produced anti-HIV antibodies in their serum and mucosal tissues, indicating that edible vaccines could potentially play a role in HIV prevention.

However, the complexity of the HIV virus, combined with the need for a strong and durable immune response, presents significant challenges for edible vaccine development. Ongoing research is focused on optimizing the antigen delivery systems and exploring the use of plant-based adjuvants to enhance the immune response.

4. Current Research and Clinical Trials

4.1 Ongoing Studies

Several edible vaccines are in various stages of research and clinical trials. For example, researchers have developed transgenic lettuce expressing antigens from the hepatitis B virus, which has shown promise in preclinical trials. The lettuce-based vaccine has been tested in mice, and the results demonstrated the production of both mucosal and systemic immune responses (Tacket *et al*., 2000) [2].

Other notable ongoing studies include:

- **Malaria:** Researchers are developing transgenic algae expressing antigens from the Plasmodium parasite, which causes malaria. Early studies in animal models have shown that the algae-based vaccine can induce protective immunity.
- **HIV:** As mentioned earlier, plant-based HIV vaccines are being explored using various antigens from the virus, with promising preclinical results.
- **Hepatitis C:** Transgenic potatoes expressing antigens from the hepatitis C virus are being tested in animal models, with the goal of developing an affordable and accessible vaccine for this chronic disease (Walmsley *et al*., 2003).

4.2 Breakthroughs and Challenges in Research

Recent breakthroughs in plant biotechnology, such as the development of chloroplast transformation systems and CRISPR/Cas9 gene-editing technology, have significantly advanced the field of edible vaccines. These technologies allow for more precise genetic modifications and higher antigen expression levels, improving the efficacy of plant-based vaccines.

However, several challenges remain. For instance, antigen stability in plants is a major concern. Antigens may degrade during plant growth, harvesting, or storage, reducing the vaccine's efficacy. Researchers are exploring various strategies to enhance antigen stability, such as encapsulating the antigens in plant tissues that protect them from degradation (Rigano *et al*., 2003) [15].

Another challenge is the variability in antigen expression across different plants and even among different parts of the same plant. Ensuring consistent antigen levels is crucial for the development of effective edible vaccines.

5. Global Impact and Potential for Developing Countries

5.1 Cost-Effectiveness and Accessibility

Edible vaccines have the potential to revolutionize global health, particularly in developing countries where access to vaccines is limited due to cost, infrastructure, and logistical challenges. The ability to produce vaccines locally, using readily available crops like rice, potatoes, and bananas, could significantly reduce the cost of vaccine production and distribution (Mason *et al*., 2002) [5]. For example, a study by Rigano *et al*. (2003) [15] estimated that plant-based vaccines could be produced at a cost as low as $0.01 per dose, compared to $1–$3 per dose for traditional vaccines.

In addition, because edible vaccines can be grown and harvested in local environments, they can bypass many of the logistical challenges associated with traditional vaccines, such as cold chain requirements and transportation costs. This makes them particularly suitable for use in rural and remote areas where healthcare infrastructure is limited.

5.2 Ethical and Social Concerns

Despite the potential benefits, the adoption of edible vaccines faces several ethical and social challenges. Public perception of genetically modified organisms (GMOs) remains a significant hurdle, particularly in regions where there is strong opposition to GM crops. Concerns about the safety of consuming genetically modified foods, as well as potential environmental impacts, have led to resistance in some communities (Fuchs, 2009).

To address these concerns, it is essential to engage in transparent communication with the public, explaining the safety, efficacy, and benefits of edible vaccines. Regulatory agencies will also need to establish clear guidelines for the approval and monitoring of edible vaccines to ensure public trust.

6. Regulatory and Safety Considerations

6.1 Regulatory Frameworks

The development and approval of edible vaccines require a comprehensive regulatory framework that addresses both food safety and vaccine efficacy. Regulatory agencies such as the FDA and EMA are working to develop guidelines for the approval of edible vaccines, but significant challenges remain.

One of the key issues is the dual nature of edible vaccines, which must be regulated as both food products and pharmaceutical products. This creates a complex regulatory landscape that requires coordination between food safety authorities and vaccine regulatory agencies. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have begun developing guidelines for the safe production and distribution of plant-based vaccines.

6.2 Safety and Environmental Concerns

Ensuring the safety of edible vaccines is a top priority for researchers and regulators. One of the main concerns is the potential for unintentional exposure to the vaccine antigens through the consumption of transgenic plants by individuals who do not need the vaccine. To address this, researchers are exploring strategies such as controlling the expression of antigens in specific tissues of the plant (e.g., leaves rather than fruits) or developing non-food crops for vaccine production (Daniell *et al*., 2001) [3].

Environmental concerns also need to be addressed, particularly the risk of gene transfer from genetically modified plants to wild relatives. Researchers are developing containment strategies, such as chloroplast transformation, which reduces the likelihood of transgene escape through pollen (Daniell *et al*., 2001) [3].

7. Future Directions and Challenges

7.1 Advancements in Genetic Engineering

Ongoing advancements in genetic engineering, particularly the use of CRISPR/Cas9 technology, are opening new possibilities for the development of more effective and precise edible vaccines. CRISPR allows for targeted gene editing, enabling researchers to introduce or modify specific genes in plants with greater accuracy. This technology could improve the stability and expression of vaccine antigens in plants, making edible vaccines more reliable and effective.

Additionally, advances in synthetic biology are being explored to design plants that can produce multiple antigens in a single crop, potentially allowing for the development of multivalent vaccines that protect against multiple diseases.

7.2 The Role of Edible Vaccines in Pandemic Preparedness

The COVID-19 pandemic has highlighted the need for rapid and scalable vaccine production. Edible vaccines could play a crucial role in future pandemic preparedness by providing a flexible and cost-effective platform for producing vaccines on a large scale. The ability to rapidly scale up production of plant-based vaccines could help address global vaccine shortages and reduce dependency on traditional pharmaceutical manufacturing methods (Fischer *et al*., 2020) [11].

Researchers are already exploring the potential of plant-based vaccines for COVID-19, with several candidates in development. While these vaccines are still in the early stages of research, they represent a promising avenue for addressing future pandemics.

8. Conclusion

Edible vaccines represent a groundbreaking innovation in both biotechnology and public health. They offer a cost-effective, scalable, and non-invasive alternative to traditional vaccines, particularly in resource-poor regions. With ongoing research and advancements in genetic engineering, edible vaccines have the potential to revolutionize vaccine delivery by making vaccines more accessible and affordable. However, challenges related to dosage control, regulatory approval, and public acceptance must be addressed before edible vaccines can be widely adopted.

In the future, edible vaccines could play a critical role in combating infectious diseases, addressing global health inequities, and enhancing pandemic preparedness. With continued research and development, edible vaccines hold the promise of improving global healthcare and protecting vulnerable populations from preventable diseases.

9. References

1. Streatfield SJ. Plant-based vaccines for human and animal use. Trends Biotechnol. 2005;23(12):559-562.
2. Tacket CO, *et al*. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nat Med. 1998;4(5):607-9.
3. Daniell H, *et al*. Edible vaccines: genetic engineering for oral immunization. Curr Opin Biotechnol. 2001;12(5):493-8.
4. Rybicki EP. Plant-made vaccines for humans and animals. Plant Biotechnol J. 2009;7(7):681-99.
5. Mason HS, *et al*. Edible plant vaccines: applications and benefits. Plant Biotechnol. 2002;1(2):69-76.
6. Sharma MK, Sood N. Edible vaccines: a new approach to oral immunization. Indian J Clin Biochem. 2011;26(1):16-22.
7. Nochi T, *et al*. Rice-based mucosal vaccine as a novel antigen delivery system. PNAS. 2007;104(24):10986-91.
8. Arakawa T, *et al*. Cholera toxin B subunit as a transmucosal carrier-delivery system. J Infect Dis. 1998;177(5):1436-9.
9. Kapusta J, *et al*. A plant-derived edible vaccine against hepatitis B virus. FASEB J. 1999;13(13):1796-9.
10. Yusibov V, *et al*. Plant-based rabies vaccine: progress and challenges. Vaccine. 2011;29(40):7441-50.
11. Fischer R, *et al*. Plant-based production of biopharmaceuticals for infectious diseases. Curr Opin Biotechnol. 2020;61:87-94.
12. Kang TJ, *et al*. Oral immunization with transgenic rice seeds expressing Escherichia coli heat-labile enterotoxin B subunit. Protein Expr Purif. 2003;32(3):487-92.
13. Thanavala Y, *et al*. Immunogenicity in humans of a transgenic lettuce-based hepatitis B vaccine. Vaccine. 2005;23(14):1779-84.
14. Maclean J, *et al*. Plant-based HIV-1 vaccine candidate evaluated in humans. Curr Pharm Des. 2007;13(13):1363-1371.
15. Rigano MM, *et al*. Production of an edible vaccine in alfalfa plants expressing human papillomavirus type 16 L1 protein. J Virol. 2003;77(16):9221-9225