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Edible vaccines from leafy vegetables: Advances in food technology, phytochemical composition and human health

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Abstract

Edible vaccines have emerged as a promising alternative to conventional vaccination systems due to their cost-effectiveness, ease of administration and potential for large-scale production. Among various plant-based platforms, leafy vegetables are considered highly suitable for edible vaccine development because of their rapid growth, high biomass production, ease of cultivation and suitability for raw consumption. This review highlights the potential of leafy vegetables as edible vaccine platforms with emphasis on food technology, phytochemical composition and human health applications. Edible vaccines are developed by introducing pathogen-specific antigen genes into edible plant tissues through genetic engineering approaches. Upon oral consumption, these antigens stimulate mucosal and systemic immune responses, providing protection against infectious diseases. Leafy vegetables such as lettuce, spinach, cabbage, kale, mustard greens and amaranthus have been extensively investigated for the production of vaccine antigens against diseases including hepatitis B, cholera, influenza, COVID-19 and norovirus. In addition to their role in antigen delivery, leafy vegetables contain important phytochemicals such as phenolics, flavonoids, carotenoids, glucosinolates, terpenoids and alkaloids that exhibit antioxidant, anti-inflammatory, antimicrobial and immunomodulatory activities. These bioactive compounds may enhance vaccine efficacy and contribute to overall health improvement.

The review also discusses the importance of food technology approaches including freeze drying, encapsulation, spray drying and nanoformulation for improving antigen stability, shelf-life and oral delivery efficiency. Recent advances in biotechnology such as chloroplast transformation, CRISPR/Cas genome editing, synthetic biology, nanobiotechnology and artificial intelligence-based omics approaches have further strengthened edible vaccine research and development. Although, challenges related to dosage consistency, biosafety, regulatory approval and public acceptance remain, leafy vegetable-based edible vaccines hold significant potential for future applications in human and veterinary healthcare, particularly in low-resource settings and mass immunization programs.

1. Introduction

Vaccination is widely recognized as one of the most effective medical interventions for preventing infectious diseases and improving global public health. Conventional vaccines have played a crucial role in reducing mortality and morbidity associated with diseases such as polio, hepatitis, measles, influenza and diphtheria. However, despite remarkable progress in vaccine development and immunization programs, traditional vaccine systems continue to face numerous limitations, including high production costs, dependence on cold-chain transportation, contamination risks, limited accessibility in rural regions and the requirement for trained healthcare professionals for administration. These challenges are particularly severe in developing countries where healthcare infrastructure and vaccine storage facilities remain inadequate. Consequently, researchers have increasingly focused on developing alternative vaccine platforms that are economical, safe, easily administrable and suitable for mass immunization programs. In this context, edible vaccines have emerged as a revolutionary innovation that integrates plant biotechnology, immunology, agriculture and food technology to create sustainable oral immunization systems (Shahid and Daniell, 2016; Rosales-Mendoza *et al*., 2020). Recent studies have highlighted the growing importance of edible vaccines as next-generation therapeutic and preventive healthcare tools capable of addressing many of the shortcomings associated with conventional vaccination strategies (Mishra *et al*., 2023). The concept of edible vaccines involves the use of genetically engineered edible plant tissues capable of producing

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antigenic proteins that stimulate immune responses when consumed orally. In edible vaccine systems, genes encoding specific pathogen antigens are inserted into plant genomes through recombinant DNA technology. These genetically modified plants subsequently synthesize antigenic proteins within edible tissues such as leaves, fruits, seeds, or tubers. Upon consumption, the antigens are recognized by the human immune system, triggering mucosal and systemic immune responses that provide protection against infectious diseases (Rosales-Mendoza *et al*., 2020). Unlike injectable vaccines, edible vaccines eliminate the need for needles, syringes and sophisticated medical facilities, thereby offering a simple and non-invasive approach to immunization. This technology has gained substantial scientific interest because it combines the nutritional value of edible plants with therapeutic immunological functions, creating dual-purpose functional foods with significant public health potential (Kumar *et al*., 2022).

The historical development of edible vaccines began during the late twentieth century when advances in plant genetic engineering enabled researchers to express foreign proteins in plant tissues. Early investigations demonstrated that transgenic plants could successfully produce bacterial and viral antigens capable of inducing immune responses in experimental animals and humans. Initial studies primarily focused on crops such as potato, tomato, tobacco, maize and banana because of their suitability for genetic transformation and large-scale cultivation. Over time, scientific attention expanded toward edible leafy vegetables owing to their rapid growth, high biomass production and suitability for raw consumption. The development of chloroplast transformation, transient expression systems and molecular farming technologies further accelerated research on plant-based vaccines (Daniell *et al*., 2019). More recently, advances in genome editing and synthetic biology have improved antigen expression efficiency and biosafety in edible crops. Current research indicates that edible vaccines could become valuable tools for combating infectious diseases, particularly in resource-limited regions where traditional vaccination systems remain inaccessible (Tiwari *et al*., 2021). Plant-based vaccines have attracted considerable attention in modern healthcare because of their ability to provide economical, scalable and environmentally sustainable vaccine production systems. Conventional vaccine manufacturing often requires expensive fermentation technologies, sterile bioreactor facilities, purification systems and refrigerated transportation. In contrast, edible vaccine production relies primarily on agricultural cultivation practices, thereby significantly reducing manufacturing and storage costs (Kwon and Daniell, 2018). Plant-derived vaccines also minimize the risk of contamination with human pathogens because plants do not support the replication of mammalian viruses and infectious agents commonly associated with animal cell culture systems. Furthermore, the oral administration of edible vaccines improves patient compliance, particularly among children and elderly populations who may experience fear or discomfort associated with injections. These advantages make edible vaccines highly suitable for large-scale immunization campaigns and emergency outbreak management (Rybicki, 2020).

One of the most important features of edible vaccines is their ability to stimulate mucosal immunity. Many infectious pathogens invade the human body through mucosal surfaces such as the gastrointestinal and respiratory tracts. Conventional injectable vaccines primarily induce systemic immune responses but may provide limited protection at mucosal entry sites. Oral edible vaccines, however, interact directly with gut-associated lymphoid tissues, leading to the activation of mucosal immune mechanisms (Rosales-Mendoza *et al*., 2020). Antigens released from plant tissues are recognized by specialized immune cells in the intestinal mucosa, stimulating the production of secretory immunoglobulin A and other protective antibodies. This dual activation of mucosal and systemic immunity enhances overall disease resistance and may provide broader protection against pathogens. Recent research involving lettuce-based oral vaccines against viral infections has demonstrated promising immunogenic responses, further supporting the role of edible vaccines in modern preventive medicine (Takeyama *et al*., 2021). The significance of plant-based vaccines became even more apparent during recent global health emergencies, including the COVID-19 pandemic. The pandemic exposed major weaknesses in existing vaccine production and distribution systems, especially in low-income countries where cold-chain logistics and manufacturing capacities were limited. Plant molecular farming emerged as an attractive alternative because plants can rapidly produce recombinant proteins and vaccine antigens at relatively low cost (Capell *et al*., 2020).

Nanotechnology-based delivery systems have also attracted considerable attention in edible vaccine research because of their ability to enhance antigen bioavailability and intestinal absorption. Nanoencapsulation techniques protect sensitive proteins from degradation caused by heat, oxygen, light and digestive enzymes. These technologies improve targeted delivery of antigens to intestinal immune cells, thereby enhancing oral vaccine efficacy (Patel *et al*., 2023). Food-grade nanoparticles, biodegradable polymers and edible coatings are increasingly being explored for stabilizing vaccine proteins and improving mucosal uptake. In addition to vaccine stabilization, food technology contributes to quality control, biosafety assurance and standardization of edible vaccine products. The implementation of hazard analysis systems and food safety regulations is essential for ensuring consumer confidence and regulatory acceptance of genetically engineered edible vaccines (Rybicki, 2020). The growing popularity of functional foods and nutraceuticals has further increased interest in edible vaccine technology. Functional foods are defined as foods that provide health benefits beyond basic nutrition, while nutraceuticals contain biologically active compounds that promote disease prevention and physiological well-being. Leafy vegetables engineered to express vaccine antigens represent a novel category of therapeutic functional foods capable of combining nutrition with immunization (Sharma *et al*., 2022). Such dual-purpose products may play a significant role in preventive healthcare strategies aimed at improving immunity and reducing disease burden. Consumer demand for natural, plant-based and health-promoting foods has increased substantially in recent years, creating favorable opportunities for the commercialization of edible vaccine products (Singh *et al*., 2023). Additionally, the incorporation of edible vaccines into commonly consumed vegetables could simplify mass immunization programs and improve vaccine accessibility in remote and economically disadvantaged populations. The present review aims to comprehensively explore the emerging role of leafy vegetables as edible vaccine platforms, with particular emphasis on advances in food technology, phytochemical composition and human health applications. The review discusses the scientific principles and historical evolution of edible vaccines, the advantages of leafy vegetables in oral immunization systems and the importance of phytochemicals in enhancing immune responses and overall health. It also examines recent innovations in food processing technologies, stabilization strategies and oral delivery systems that improve antigen stability and vaccine efficacy. By integrating concepts from plant biotechnology, food science, nutrition, immunology and public health, this review seeks to provide a comprehensive understanding of the opportunities, challenges and future prospects associated with leafy vegetable-based edible vaccines as sustainable next-generation healthcare solutions.

2. Edible vaccines: Concept and mechanism

Edible vaccines are genetically engineered plant-based vaccines in which antigenic proteins derived from pathogens are expressed in edible plant tissues and delivered orally through consumption. These vaccines are designed to stimulate protective immune responses without the use of injections or conventional pharmaceutical formulations. The development of edible vaccines represents a major advancement in plant biotechnology and molecular farming because plants can serve simultaneously as biofactories for antigen production and as delivery vehicles for oral immunization (Mason *et al*., 2021). The principle underlying edible vaccine technology involves the insertion of genes encoding specific pathogenic antigens into plant genomes using recombinant DNA techniques. Once integrated into the plant cells, these genes direct the synthesis of antigenic proteins that accumulate within edible tissues such as leaves, fruits, tubers, or seeds. Upon ingestion, the antigens are recognized by the immune system, leading to the activation of both mucosal and systemic immune responses (Waheed *et al*., 2020). The concept of oral immunization is based on the natural ability of the mucosal immune system to recognize and respond to foreign antigens entering through the gastrointestinal tract. Oral vaccines are particularly advantageous because many infectious microorganisms invade the body through mucosal surfaces, including the digestive and respiratory systems. Edible vaccines utilize this natural defense mechanism by delivering antigens directly to gut-associated lymphoid tissues. Unlike injectable vaccines that primarily stimulate circulating antibodies, oral vaccines can induce local mucosal immunity in addition to systemic protection (Hayden *et al*., 2021). This dual immune activation is considered highly beneficial for controlling enteric and respiratory infections. Moreover, oral immunization eliminates needle-associated pain, reduces the risk of infections caused by unsafe injection practices and improves patient compliance, particularly among children and elderly individuals. Antigen expression in plants is achieved through various transformation techniques such as *Agrobacterium*-mediated transformation, biolistic gene transfer, chloroplast transformation and transient expression systems. These technologies enable stable or temporary integration of foreign genes into plant cells for recombinant protein production. Among these approaches, chloroplast transformation has gained significant attention because chloroplasts can produce high levels of recombinant proteins while minimizing transgene escape through pollen transmission (Bock and Warzecha, 2019). Transient expression systems using viral vectors have also emerged as rapid and efficient methods for producing vaccine antigens in leafy vegetables within short time periods. The selection of suitable plant hosts is essential for successful edible vaccine production and leafy vegetables such as lettuce and spinach are increasingly preferred because of their rapid growth, high biomass accumulation and suitability for raw consumption (Schillberg *et al*., 2019).

The effectiveness of edible vaccines depends largely on their ability to stimulate appropriate immune responses following oral administration. After consumption of vaccine-containing plant tissues, antigenic proteins are released in the gastrointestinal tract and interact with specialized immune cells located within mucosal tissues. The intestinal mucosa serves as the first line of defense against orally encountered pathogens and contains highly organized immune structures collectively known as gut-associated lymphoid tissues (GALT). These tissues include Peyer's patches, isolated lymphoid follicles, mesenteric lymph nodes and mucosal epithelial cells that participate in antigen recognition and immune activation (Azegami *et al*., 2021). Mucosal immunity represents one of the most important immunological features of edible vaccines. Mucosal surfaces are continuously exposed to environmental microorganisms and therefore require specialized immune mechanisms to prevent infections. When edible vaccine antigens reach the intestine, they are captured by microfold (M) cells located in Peyer's patches. These M cells transport antigens across the epithelial barrier and deliver them to antigen-presenting cells such as dendritic cells and macrophages. Subsequently, these immune cells process and present the antigens to T lymphocytes, leading to the activation of B cells and antibody production (Yuki and Kiyono, 2020). One of the major outcomes of mucosal immune activation is the production of secretory immunoglobulin A (sIgA), which plays a critical role in neutralizing pathogens at mucosal surfaces before they invade host tissues. In addition to mucosal immunity, edible vaccines are capable of inducing systemic immune responses. Activated B cells and T cells generated within gut-associated lymphoid tissues migrate to systemic circulation, where they contribute to broader immune protection throughout the body. Plasma cells produce circulating immunoglobulin G (IgG) antibodies that provide long-term systemic immunity against pathogens (Kiyono and Fukuyama, 2020). This combined induction of mucosal and systemic immunity offers significant advantages over many conventional vaccines that primarily stimulate systemic antibody responses. Furthermore, oral immunization through edible vaccines may generate immunological memory, enabling rapid and effective responses upon future exposure to the pathogen. Antigen uptake within the gastrointestinal tract is a critical factor influencing the efficacy of edible vaccines. Plant cell walls composed of cellulose and lignin provide natural bioencapsulation that protects antigenic proteins from degradation in the acidic environment of the stomach. This protective effect allows intact antigens to reach the intestine where microbial enzymes gradually degrade plant cell walls and release the vaccine proteins (Kwon *et al*., 2023). The efficiency of antigen uptake may vary depending on factors such as plant species, antigen stability, dosage, food processing methods and individual digestive conditions. To improve antigen delivery and absorption, researchers have explored various formulation strategies including encapsulation, adjuvant incorporation and nanoparticle-based delivery systems (Su *et al*., 2022). These approaches enhance antigen stability and facilitate efficient interaction with mucosal immune cells.

3. Leafy vegetables as platforms for edible vaccines

3.1 Characteristics of suitable leafy vegetables

Leafy vegetables have emerged as highly promising platforms for edible vaccine production because of their favourable biological, agronomic and biotechnological characteristics. The selection of suitable host plants is one of the most important factors determining the success of edible vaccine development. An ideal plant platform should possess rapid growth, high biomass accumulation, efficient recombinant protein expression, ease of cultivation, environmental adaptability and consumer acceptability. Leafy vegetables fulfil many of these requirements and therefore represent valuable candidates for oral vaccine delivery systems (Pniewski, 2022). Their ability to produce large amounts of edible biomass within short cultivation periods enables rapid and cost-effective vaccine production, which is particularly important during disease outbreaks and public health emergencies (Figure 1).

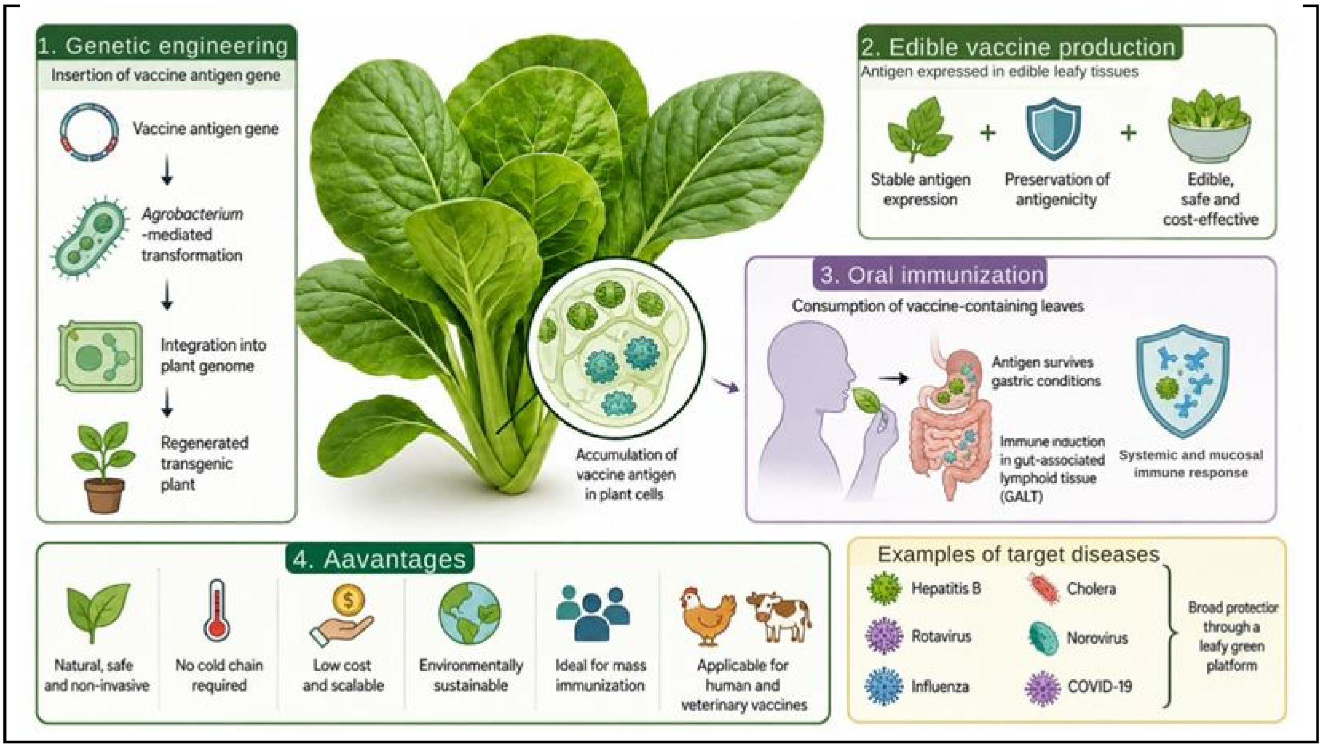

Figure 1: Schematic representation of leafy vegetables as platforms for edible vaccine production, antigen expression and oral immunization mechanisms.

Rapid biomass production is one of the major advantages of leafy vegetables used in molecular farming. Crops such as lettuce, spinach, mustard greens and kale have short life cycles and can be harvested multiple times annually, allowing continuous production of recombinant proteins. Fast-growing leafy vegetables facilitate quick scaling of vaccine production without the need for extensive infrastructure or prolonged cultivation periods (Makhzoum *et al.*, 2020). In controlled environment agriculture systems such as hydroponics and vertical farming, leafy vegetables can achieve accelerated growth rates while maintaining high-quality biomass production. This rapid biomass accumulation significantly enhances the economic feasibility of plant-based vaccine manufacturing. Another important characteristic of suitable leafy vegetables is their high protein expression capacity. Efficient antigen production is essential for achieving adequate immunogenicity in edible vaccine systems. Many leafy vegetables exhibit strong cellular metabolic activity and large photosynthetic surface areas that support enhanced recombinant protein synthesis (Buyel *et al.*, 2021). Additionally, leafy tissues are often rich in chloroplasts, which can serve as highly efficient sites for recombinant protein accumulation. Chloroplast engineering has demonstrated the ability to produce exceptionally high levels of vaccine antigens in leafy vegetables compared with many other plant organs. High protein expression capacity reduces the quantity of plant material required for immunization and improves the practicality of edible vaccine administration.

Ease of cultivation further contributes to the suitability of leafy vegetables as edible vaccine platforms. Most leafy vegetables can be cultivated using simple agricultural techniques under field, greenhouse, or indoor farming conditions. Their relatively low cultivation costs and adaptability to diverse climatic environments make them suitable for decentralized vaccine production systems in different regions of the world (Margolin *et al.*, 2020). Moreover, leafy vegetables generally require shorter postharvest processing times and can often be consumed fresh, thereby minimizing antigen degradation associated with cooking or industrial processing. Controlled cultivation systems additionally reduce contamination risks and facilitate standardized production conditions necessary for pharmaceutical applications. Leafy vegetables also possess favorable safety and consumer acceptance profiles because they are already widely consumed as part of normal human diets. Their edible nature simplifies oral vaccine administration and improves compliance among children and elderly populations. Furthermore, leafy vegetables contain natural bioencapsulation properties due to their plant cell wall structures, which protect antigenic proteins from degradation in the gastrointestinal tract (Chan and Daniell, 2022). This natural protection enhances antigen stability during digestion and improves delivery efficiency to intestinal immune tissues. Consequently, leafy vegetables provide multifunctional advantages that combine nutritional value, pharmaceutical applications and ease of production.

3.2 Major leafy vegetables used in edible vaccine research

Several leafy vegetable species have been investigated extensively for edible vaccine production because of their high transformation efficiency, rapid growth and favorable consumption characteristics. Among these, lettuce is considered one of the most successful and widely utilized host plants for oral vaccine development. Lettuce is highly preferred because it is commonly consumed raw, allowing vaccine antigens to remain biologically active without exposure to thermal degradation during cooking. In addition, lettuce exhibits efficient genetic transformation, rapid biomass production and suitability for greenhouse cultivation (Tregoning *et al*., 2020). Recombinant lettuce plants have been engineered to express antigens against cholera, hepatitis B, norovirus, influenza and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clinical and experimental studies have demonstrated that lettuce-derived vaccines can successfully induce antigen-specific immune responses following oral administration.

Spinach has also gained considerable attention as a platform for edible vaccine production because of its high nutritional value, soft leaf tissues and adaptability to different environmental conditions. Spinach leaves contain abundant vitamins, minerals, antioxidants and phytochemicals that may contribute additional health benefits alongside vaccine delivery (Nayak and Kundu, 2021). Researchers have explored spinach-based systems for expressing bacterial and viral antigens capable of inducing mucosal immunity. The high water content and digestibility of spinach facilitate antigen release in the gastrointestinal tract, enhancing oral vaccine efficiency. Furthermore, spinach can be cultivated under hydroponic systems, enabling controlled production environments for pharmaceutical applications. Cabbage represents another promising leafy vegetable for edible vaccine research. Cabbage is widely cultivated and consumed globally and possesses substantial biomass production capacity. Members of the Brassicaceae family are known for their high glucosinolate and antioxidant content, which may support immune modulation and improve vaccine functionality (Kumari *et al*., 2022). Genetic transformation studies in cabbage have demonstrated successful expression of recombinant proteins and pathogen-derived antigens. Additionally, cabbage exhibits relatively strong environmental adaptability and can be cultivated under various agroclimatic conditions, making it suitable for large-scale molecular farming applications.

Amaranthus species have recently emerged as valuable alternative platforms for edible vaccine production due to their rapid growth, nutritional richness and stress tolerance. Amaranthus leaves are highly nutritious and contain significant amounts of proteins, minerals, vitamins and bioactive phytochemicals. These plants exhibit excellent adaptability to adverse environmental conditions including drought and poor soil fertility, which may support sustainable vaccine production in resource-limited regions (Rai *et al*., 2021). Although, research on amaranthus-based edible vaccines remains relatively limited compared with lettuce and spinach, recent advancements in plant transformation technologies suggest strong potential for future applications in oral immunization systems. Mustard greens are also being explored for recombinant protein production because of their rapid biomass accumulation and high chloroplast density. Mustard plants are highly adaptable and can be cultivated under a wide range of environmental conditions. Their leaves possess strong metabolic activity and favorable characteristics for chloroplast transformation, enabling efficient accumulation of foreign proteins (Saxena *et al*., 2020). In addition, mustard greens contain important phytochemicals such as glucosinolates, flavonoids and phenolic compounds that exhibit antioxidant and immunomodulatory properties. These compounds may provide synergistic effects when combined with vaccine antigens.

Kale has gained popularity not only as a functional food but also as a potential molecular farming platform. Kale is rich in antioxidants, carotenoids, vitamins and minerals that support human health and immune function. Its robust leaf structure and high biomass productivity make it suitable for recombinant protein production (Kyriakopoulou *et al*., 2021). Moreover, kale can be cultivated in controlled environments with minimal resource requirements, contributing to sustainable edible vaccine production systems. The increasing popularity of kale in health-conscious diets may further improve consumer acceptance of vaccine-containing functional foods derived from leafy vegetables.

4. Food technology approaches in edible vaccine development

4.1 Processing technologies

Food technology plays a crucial role in the successful development, stabilization, storage and delivery of edible vaccines. Although, plant-based vaccine systems offer several advantages over conventional vaccines, maintaining antigen integrity and biological activity during processing and storage remains a significant challenge. Recombinant proteins expressed in leafy vegetables are often sensitive to environmental conditions such as heat, moisture, oxygen and enzymatic degradation. Therefore, advanced food processing technologies are required to preserve antigen stability and enhance vaccine efficacy. Modern food engineering approaches including freeze drying, encapsulation, spray drying and nanoformulation techniques have significantly improved the feasibility of edible vaccine systems for commercial and pharmaceutical applications (Pérez Aguirre-burualde *et al*., 2022). Freeze drying, also known as lyophilization, is one of the most widely used preservation methods in edible vaccine development. This technique involves the removal of water from plant tissues under low temperature and vacuum conditions, thereby minimizing thermal damage to sensitive antigenic proteins. Freeze drying preserves the structural and functional integrity of recombinant proteins while extending product shelf life (Islam *et al*., 2021). In edible vaccine systems, freeze-dried leafy vegetables such as lettuce and spinach can retain antigen stability for prolonged periods under ambient conditions. The reduction in moisture content also decreases microbial growth and enzymatic degradation, thereby improving storage safety. Furthermore, lyophilized vaccine-containing plant materials can be converted into powders or capsules, facilitating easier dosage standardization and transportation. Several studies have demonstrated that freeze-dried plant tissues maintain immunogenic activity even after long-term storage, highlighting the importance of this technology in plant-based vaccine preservation (Figure 2).

Encapsulation technologies have also gained considerable importance in edible vaccine development because of their ability to protect antigenic proteins from degradation during gastrointestinal digestion. Encapsulation involves enclosing vaccine antigens within protective matrices composed of biopolymers, lipids, proteins, or polysaccharides (de Oliveira *et al*., 2020). This protective barrier shields antigens from acidic gastric conditions and digestive enzymes, allowing controlled release within the intestine where immune activation occurs. Encapsulation additionally improves antigen stability during storage and enhances mucosal absorption efficiency. Natural food-grade materials such as alginate, chitosan, starch, gelatin and pectin are commonly used for encapsulating edible vaccine components. Microencapsulation and nanoencapsulation techniques are particularly effective in improving oral bioavailability and targeted antigen delivery to gut-associated lymphoid tissues. Spray drying is another important food processing technology applied in edible vaccine formulation. This method converts liquid plant extracts or recombinant protein suspensions into stable dry powders through rapid evaporation under controlled conditions. Spray drying offers advantages such as rapid processing, scalability, low operational costs and improved product stability (Jafari *et al*., 2021). Although, high temperatures used during spray drying may potentially affect sensitive proteins, optimization of processing parameters and incorporation of stabilizing agents can significantly reduce antigen degradation. Spray-dried edible vaccine powders exhibit reduced moisture content, enhanced portability and easier incorporation into food products or oral dosage forms. This technology is particularly useful for developing stable vaccine formulations suitable for mass distribution in regions lacking refrigeration facilities.

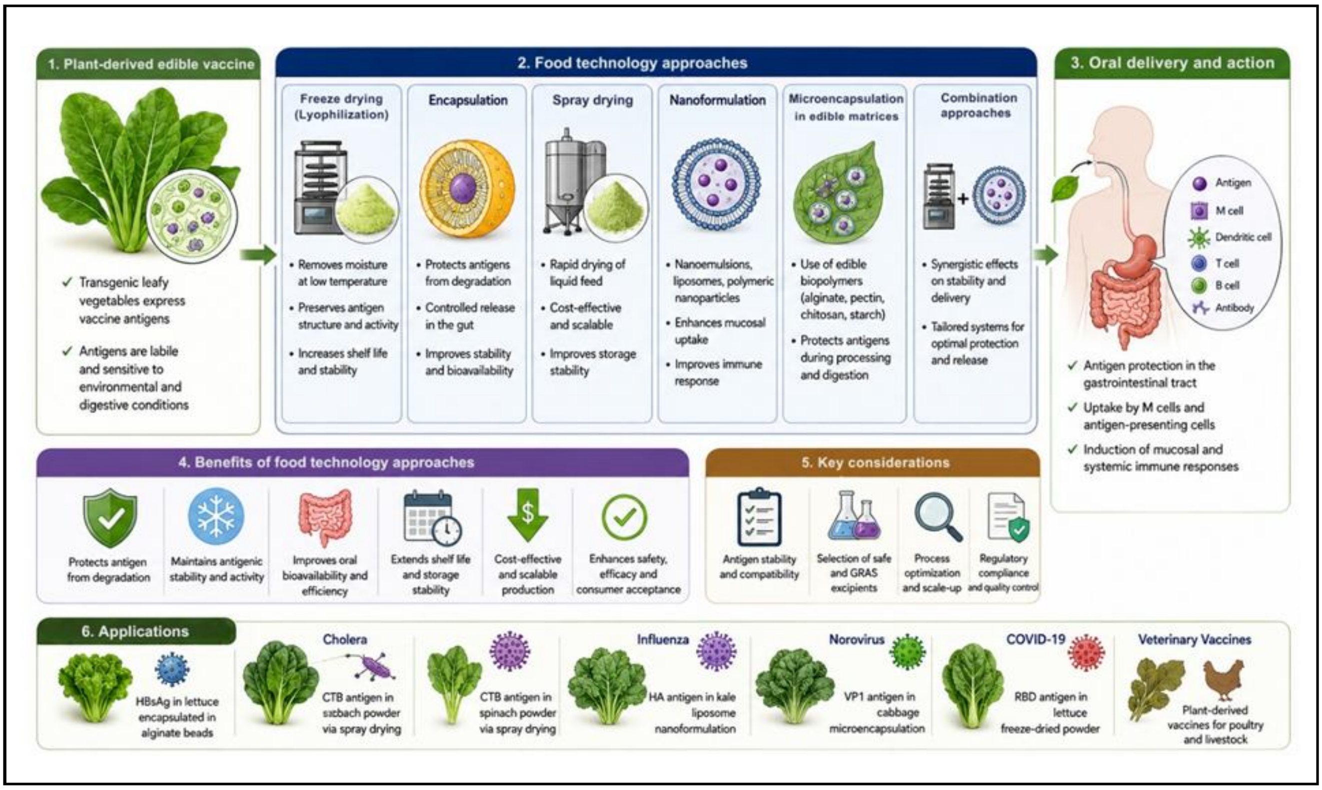

Figure 2: Food technology approaches in edible vaccine development: Processing technologies, antigen stabilization and oral delivery mechanisms for plant-derived vaccines.

Nanoformulation approaches have emerged as advanced strategies for improving edible vaccine delivery and immunogenicity. Nanotechnology-based delivery systems utilize nanoparticles, nanoemulsions, liposomes and nanofibers to enhance antigen protection, controlled release and intestinal uptake (Chavda *et al*., 2023). Nanoparticles provide large surface area-to-volume ratios that facilitate efficient interaction with mucosal immune cells. Moreover, nanoformulations improve antigen stability against environmental stressors such as heat, oxidation and enzymatic degradation. Lipid-based nanoparticles and biodegradable polymeric nanocarriers have shown promising results in enhancing oral vaccine bioavailability and immune stimulation. Nanoencapsulation also enables co-delivery of adjuvants and bioactive phytochemicals that may further strengthen immune responses. Recent research indicates that nanoformulation technologies may significantly improve the therapeutic performance and commercial viability of edible vaccines.

4.2 Food safety and quality control

Food safety and quality control are fundamental components of edible vaccine production because these products are intended for direct human consumption. Unlike conventional pharmaceutical vaccines, edible vaccines must satisfy both food safety regulations and biopharmaceutical quality standards. Ensuring the absence of microbial contamination, toxins, allergens and unintended genetic effects is critical for consumer safety and public acceptance (Fahad *et al*., 2021). Therefore, stringent quality assurance systems and biosafety protocols are necessary throughout cultivation, processing, storage and distribution stages. Contamination prevention is one of the most important aspects of edible vaccine manufacturing. Plant materials may be exposed to microbial pathogens, environmental pollutants, pesticides and cross-contamination during cultivation and processing. Controlled environment agriculture systems such as greenhouses, hydroponics and vertical farms are increasingly used to minimize contamination risks and maintain standardized production conditions (Mitra *et al*., 2022). Sterile cultivation techniques, water quality management and hygienic handling practices are essential for ensuring product safety. Furthermore, recombinant plants intended for vaccine production are often cultivated in isolated environments to prevent unintended gene transfer to surrounding crops or ecosystems.

Standardization protocols are equally important in maintaining consistency and reproducibility in edible vaccine products. Variability in antigen expression levels among plant tissues can result in inconsistent immune responses and reduced vaccine efficacy. Standardized cultivation practices, genetic stability assessments and quality control testing are therefore required to ensure uniform antigen concentrations (Hefferon, 2020). Analytical techniques such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), western blotting and chromatographic methods are commonly used for quantifying recombinant proteins and verifying product quality. Standard operating procedures and good manufacturing practices (GMP) further contribute to maintaining product consistency and regulatory compliance. Biosafety measures are essential for addressing concerns related to genetically modified organisms (GMOs) and environmental impacts. Regulatory agencies require comprehensive biosafety evaluations before approving edible vaccine products for human use. These evaluations include assessments of allergenicity, toxicity, gene stability, environmental release risks and potential ecological impacts (Spök *et al*., 2022). Chloroplast transformation technologies are often preferred because they reduce transgene escape through pollen transmission, thereby improving environmental containment. Additionally, marker-free transformation systems and gene-editing technologies are being explored to enhance biosafety and public acceptance of plant-derived vaccines.

4.3 Functional food and nutraceutical perspectives

The increasing demand for functional foods and nutraceuticals has created new opportunities for integrating edible vaccines into health-promoting dietary systems. Functional foods are foods that provide physiological and health benefits beyond basic nutrition, while nutraceuticals are products containing bioactive compounds capable of preventing or managing diseases (Granato *et al*., 2020). Edible vaccines derived from leafy vegetables represent an innovative category of therapeutic functional foods because they combine nutritional value with immunological protection. This dual functionality makes edible vaccines particularly attractive for preventive healthcare and wellness-oriented food industries. Integration with functional foods offers several advantages for edible vaccine delivery. Leafy vegetables used in vaccine production naturally contain vitamins, minerals, antioxidants, flavonoids, carotenoids and dietary fibers that contribute to human health and immune support (Prokopov *et al*., 2021). These bioactive phytochemicals may synergistically enhance vaccine efficacy by modulating immune responses and reducing oxidative stress. Consequently, edible vaccines can provide simultaneous nutritional supplementation and immunization benefits. The incorporation of vaccine-containing leafy vegetable powders into beverages, smoothies, soups, energy bars and dietary supplements may further expand their practical applications and consumer acceptance.

Commercial food product development involving edible vaccines is an emerging area within food biotechnology and pharmaceutical industries. Advances in food processing, encapsulation and formulation technologies have enabled the development of stable vaccine-containing products with improved sensory quality and shelf life (Kowalski *et al*., 2022). Freeze-dried powders, capsules, tablets, oral films and fortified food products are being investigated as potential delivery formats for edible vaccines. Consumer-friendly formulations may improve compliance and facilitate integration into daily dietary practices. The commercialization potential of edible vaccines is particularly significant in developing countries where malnutrition and infectious diseases often coexist. Functional foods containing vaccine antigens and essential nutrients could simultaneously address nutritional deficiencies and disease prevention (Makkar *et al*., 2021). Moreover, the growing popularity of plant-based diets and natural health products provides favorable market conditions for edible vaccine products derived from leafy vegetables.

5. Phytochemical composition of leafy vegetables

5.1 Major phytochemicals

Leafy vegetables are recognized as rich sources of diverse phytochemicals that contribute significantly to human nutrition, disease prevention and overall health promotion. Phytochemicals are naturally occurring bioactive compounds synthesized by plants as part of their defense mechanisms against environmental stresses, pathogens, ultraviolet radiation and herbivores. In edible vaccine systems, these phytochemicals are particularly important because they not only provide nutritional and therapeutic benefits but may also enhance immune responses and improve vaccine efficacy. Leafy vegetables such as lettuce, spinach, kale, mustard greens, cabbage and amaranthus contain a broad spectrum of phytochemicals including phenolic compounds, flavonoids, alkaloids, carotenoids, terpenoids and glucosinolates (Li *et al*., 2022). These compounds exhibit antioxidant, anti-inflammatory, antimicrobial and immunomodulatory activities that support human health and may synergistically interact with vaccine antigens in plant-based oral immunization systems. Phenolic compounds represent one of the largest and most important groups of phytochemicals present in leafy vegetables. These compounds are characterized by the presence of one or more hydroxyl groups attached to aromatic rings and include phenolic acids, tannins, lignans and polyphenols. Common phenolic compounds identified in leafy vegetables include caffeic acid, ferulic acid, chlorogenic acid, gallic acid and coumaric acid (Dias *et al*., 2021). Phenolics play crucial roles in protecting plant tissues against oxidative stress and environmental damage. In human health, phenolic compounds exhibit strong antioxidant properties by scavenging free radicals and reducing oxidative stress-related cellular damage. Moreover, phenolics contribute to anti-inflammatory, anticancer, cardioprotective and neuroprotective activities. Their antioxidant properties may also help stabilize recombinant proteins and vaccine antigens within edible vaccine systems, thereby improving oral immunization efficiency.

Flavonoids are another major class of phytochemicals abundantly found in leafy vegetables. Structurally, flavonoids consist of polyphenolic compounds categorized into flavonols, flavones, flavanones, anthocyanins and catechins. Common flavonoids present in leafy vegetables include quercetin, kaempferol, luteolin, apigenin and rutin (Borges *et al*., 2020). Flavonoids contribute significantly to the color, flavor and defense mechanisms of plants. In humans, they exhibit potent antioxidant and anti-inflammatory properties and are known to modulate immune responses, enzyme activities and cellular signaling pathways. Several studies have demonstrated that flavonoids can enhance immune cell activation, regulate cytokine production and improve resistance against infections. These immunomodulatory effects may provide synergistic advantages when leafy vegetables are used as edible vaccine carriers. Alkaloids are nitrogen-containing bioactive compounds synthesized by plants primarily as protective metabolites against herbivores and microbial pathogens. Although, leafy vegetables generally contain lower alkaloid concentrations compared with medicinal plants, several important alkaloids have been identified in spinach, mustard greens and amaranthus species (Aloo *et al*., 2021). Alkaloids possess diverse pharmacological properties including antimicrobial, analgesic, anti-inflammatory and anticancer activities. Certain alkaloids may influence immune function by modulating inflammatory mediators and immune signaling pathways. However, the concentration and safety of alkaloids must be carefully monitored because excessive intake of some alkaloids may produce toxic effects. In edible vaccine systems, understanding alkaloid composition is important for ensuring both therapeutic efficacy and food safety.

Carotenoids are lipid-soluble pigments responsible for the yellow, orange and red coloration of many plant tissues. Leafy vegetables are rich sources of carotenoids such as β-carotene, lutein, zeaxanthin and lycopene (Saini *et al*., 2022). These compounds play essential roles in photosynthesis and photoprotection within plant cells. In human nutrition, carotenoids function as precursors of vitamin A and exhibit strong antioxidant properties. They contribute to eye health, immune function, cardiovascular protection and reduction of oxidative stress. Lutein and zeaxanthin are particularly important for retinal protection and prevention of age-related macular degeneration. Recent studies have also indicated that carotenoids can enhance immune responses by stimulating lymphocyte proliferation and regulating inflammatory pathways. Therefore, carotenoid-rich leafy vegetables may provide additional health benefits in edible vaccine applications. Terpenoids constitute one of the most structurally diverse groups of phytochemicals found in plants. Derived from isoprene units, terpenoids include monoterpenes, diterpenes, triterpenes and sesquiterpenes with various biological functions. Leafy vegetables contain numerous terpenoids involved in plant defense, aroma formation and stress tolerance (Singh *et al*., 2021). In human health, terpenoids exhibit antimicrobial, antiviral, anti-inflammatory, antioxidant and anticancer activities. Certain terpenoids have been reported to enhance immune responses and improve resistance against microbial infections. Additionally, terpenoids may contribute to the stability and bioavailability of recombinant proteins within plant tissues, supporting their potential role in edible vaccine systems.

Glucosinolates are sulfur-containing secondary metabolites predominantly found in cruciferous leafy vegetables such as cabbage, kale, mustard greens and broccoli. These compounds are hydrolyzed by the enzyme myrosinase to produce biologically active isothiocyanates and indoles when plant tissues are damaged or consumed (Bell *et al*., 2018). Glucosinolates and their metabolites possess strong antioxidant, antimicrobial, anti-inflammatory and anticancer properties. Sulforaphane, one of the most extensively studied isothiocyanates derived from glucosinolates, has demonstrated significant immunomodulatory and chemoprotective effects. The presence of glucosinolates in leafy vegetables may therefore enhance the therapeutic value of edible vaccines by supporting immune health and disease prevention.

5.2 Nutritional composition

In addition to their phytochemical diversity, leafy vegetables possess exceptional nutritional value and are considered essential components of balanced human diets. Their high concentrations of vitamins, minerals, dietary fiber and essential amino acids contribute significantly to metabolic functions, immune health and disease prevention. The nutritional richness of leafy vegetables further strengthens their suitability as edible vaccine platforms because they can simultaneously provide nutritional supplementation and immunological protection (Gomes *et al*., 2021). Leafy vegetables are excellent sources of vitamins, particularly vitamins A, C, E and K, as well as several B-complex vitamins. Vitamin A, primarily present in the form of β-carotene, plays a crucial role in vision, immune function and epithelial tissue maintenance. Vitamin C acts as a powerful antioxidant that supports collagen synthesis, immune defense and iron absorption. Vitamin E contributes to cellular membrane protection against oxidative stress, while vitamin K is essential for blood coagulation and bone metabolism (Kyriacou *et al*., 2021). Folate, riboflavin, niacin and pyridoxine present in leafy vegetables participate in numerous metabolic and enzymatic reactions necessary for energy production and cellular function. These vitamins collectively enhance nutritional status and may improve immune responsiveness in individuals consuming edible vaccine-containing vegetables.

Minerals present in leafy vegetables are equally important for human health and physiological functions. Common minerals found in spinach, kale, amaranthus and mustard greens include calcium, iron, magnesium, potassium, phosphorus, zinc and selenium (Moyo and Aremu, 2022). Calcium is essential for bone health and muscle function, while iron supports oxygen transport and hemoglobin synthesis. Magnesium participates in enzymatic reactions and energy metabolism and potassium regulates electrolyte balance and cardiovascular function. Zinc and selenium contribute significantly to immune regulation and antioxidant defense systems. Adequate mineral intake through leafy vegetables may therefore strengthen immune competence and support the effectiveness of oral vaccine systems. Dietary fiber represents another major nutritional component of leafy vegetables. Plant fibers include soluble and insoluble polysaccharides such as cellulose, hemicellulose, pectin and lignin that contribute to gastrointestinal health and metabolic regulation. Dietary fiber improves digestion, supports beneficial gut microbiota, regulates blood glucose levels and reduces cholesterol absorption (Dhingra *et al*., 2022). In edible vaccine systems, plant cell wall fibers additionally provide natural bioencapsulation that protects antigenic proteins during gastric passage and facilitates controlled antigen release in the intestine. The interaction between dietary fibers and gut microbiota may further influence mucosal immunity and enhance vaccine responsiveness.

Leafy vegetables also contain essential amino acids and plant proteins required for growth, tissue repair, enzyme synthesis and immune function. Although, the protein content of leafy vegetables is generally lower than legumes and animal products, species such as amaranthus and spinach contain relatively high levels of lysine, leucine, methionine, threonine and valine (Uusiku *et al*., 2020). These amino acids contribute to immune cell proliferation, antibody production and maintenance of physiological homeostasis. Protein-rich leafy vegetables may therefore provide additional nutritional advantages when used as edible vaccine carriers.

5.3 Role of phytochemicals in human health

Phytochemicals present in leafy vegetables play fundamental roles in promoting human health and preventing chronic diseases. These bioactive compounds interact with various cellular, biochemical and molecular pathways to exert antioxidant, anti-inflammatory, antimicrobial and immunomodulatory effects. In edible vaccine systems, phytochemicals may additionally contribute to improved vaccine stability, enhanced immune responses and overall therapeutic efficacy (Sharma *et al*., 2021). One of the most important biological functions of phytochemicals is antioxidant activity. Reactive oxygen species and free radicals generated during normal metabolism or environmental stress can cause oxidative damage to lipids, proteins and DNA, contributing to aging and numerous chronic diseases. Phenolics, flavonoids, carotenoids and glucosinolates found in leafy vegetables possess strong antioxidant properties capable of neutralizing free radicals and reducing oxidative stress (Kaur *et al*., 2022). Antioxidant phytochemicals protect cellular structures from oxidative injury and support the maintenance of physiological homeostasis. In edible vaccine systems, antioxidants may also preserve antigen integrity and reduce oxidative degradation during processing and storage. Phytochemicals additionally exhibit significant anti-inflammatory effects by modulating inflammatory mediators and signaling pathways. Chronic inflammation is associated with cardiovascular diseases, diabetes, neurodegenerative disorders, obesity and cancer. Bioactive compounds such as quercetin, kaempferol, sulforaphane and lutein can suppress pro-inflammatory cytokines, inhibit cyclooxygenase enzymes and regulate nuclear factor-kappa B signaling pathways (Ahmed and Eun, 2020). These anti-inflammatory properties contribute to improved immune regulation and disease prevention. Furthermore, reducing excessive inflammation may improve vaccine tolerance and support balanced immune responses.

Antimicrobial properties of phytochemicals are also widely recognized. Many compounds present in leafy vegetables possess antibacterial, antiviral, antifungal and antiparasitic activities. Glucosinolates, phenolics, terpenoids and alkaloids can inhibit microbial growth through disruption of cell membranes, enzyme inhibition and interference with microbial metabolism (Panche *et al*., 2021). Such antimicrobial effects contribute to food preservation, gastrointestinal health and protection against infectious diseases. In edible vaccine systems, antimicrobial phytochemicals may help reduce microbial contamination and support overall product safety. The immunomodulatory potential of phytochemicals represents one of the most important aspects of their role in edible vaccines. Several phytochemicals have been shown to stimulate immune cell proliferation, regulate cytokine secretion, enhance macrophage activity and improve antibody responses (Ganesan and Xu, 2021). Flavonoids and carotenoids can influence T-cell and B-cell functions, while glucosinolate metabolites may activate antioxidant and detoxification pathways involved in immune regulation. These immunomodulatory activities may synergistically enhance the efficacy of vaccine antigens expressed in leafy vegetables.

6. Interaction between phytochemicals and vaccine efficacy

6.1 Immunomodulatory effects of phytochemicals

Phytochemicals present in leafy vegetables play a significant role in modulating immune responses and may substantially influence the efficacy of edible vaccines. These naturally occurring bioactive compounds interact with various components of the innate and adaptive immune systems, thereby enhancing antigen recognition, immune signaling and antibody production. The combination of vaccine antigens with immunomodulatory phytochemicals creates a multifunctional oral immunization system capable of providing both therapeutic and nutritional benefits (Zhao *et al*., 2021). Recent research has increasingly focused on understanding the mechanisms through which phytochemicals improve vaccine-induced immunity, particularly in plant-based oral vaccine platforms. One of the most important immunological functions of phytochemicals is the enhancement of mucosal immunity. Since edible vaccines are administered orally, the stimulation of mucosal immune responses within the gastrointestinal tract is essential for effective protection against pathogens. Phytochemicals such as flavonoids, carotenoids, phenolic acids and glucosinolate derivatives can positively influence gut-associated lymphoid tissues and intestinal immune signaling pathways (Wang *et al*., 2022). These compounds promote the activation of macrophages, dendritic cells, T lymphocytes and B lymphocytes involved in mucosal defense mechanisms. Enhanced production of secretory immunoglobulin A (sIgA) has been observed in response to phytochemical-rich diets, suggesting improved mucosal barrier protection against microbial pathogens. Phytochemicals may also influence gut microbiota composition, which plays a crucial role in immune regulation and vaccine responsiveness. Beneficial intestinal microorganisms contribute to mucosal barrier integrity, cytokine balance and immune maturation. Dietary polyphenols and fibers present in leafy vegetables can promote the growth of beneficial probiotic bacteria while suppressing harmful pathogens (Markowiak and Slizewska, 2021). Improved gut microbial balance may enhance antigen uptake and mucosal immune responses following edible vaccine consumption.

6.2 Synergistic health benefits

One of the unique advantages of leafy vegetable-based edible vaccines is their ability to provide synergistic health benefits through the integration of nutritional, phytochemical and immunological functions. Unlike conventional vaccines that primarily focus on disease prevention, edible vaccines derived from nutrient-rich leafy vegetables can simultaneously deliver essential nutrients, bioactive phytochemicals and protective antigens (Verma *et al*., 2022). This combination creates multifunctional therapeutic foods capable of supporting overall health, enhancing immune function and reducing the risk of infectious and chronic diseases. The combined nutraceutical and immunization effects of edible vaccines are particularly important in populations suffering from malnutrition, micronutrient deficiencies and weakened immune systems. Nutritional deficiencies often impair immune competence and reduce vaccine responsiveness, especially in children and elderly individuals. Leafy vegetables naturally contain vitamins, minerals, antioxidants, dietary fibers and essential amino acids that contribute to improved nutritional status and immune health (Maldonado-Celis *et al*., 2019). For example, vitamins A, C and E support antioxidant defense systems and immune cell function, while minerals such as zinc, selenium and iron are essential for antibody production and cellular immunity. By integrating these nutrients with vaccine antigens, edible vaccines may improve both nutritional rehabilitation and immunological protection simultaneously. Leafy vegetable-based edible vaccines may therefore function as integrated therapeutic systems combining the benefits of nutraceuticals, functional foods and immunization technologies. Such multifunctional products could play an important role in preventive healthcare strategies aimed at reducing disease burden and improving public health outcomes. The growing consumer interest in natural health products and plant-based functional foods further supports the commercial potential of edible vaccines with synergistic nutritional and immunological properties.

6.3 Influence of processing on phytochemicals and antigen stability

Food processing methods significantly influence the stability, bioavailability and biological activity of both phytochemicals and vaccine antigens present in edible vaccine systems. Since recombinant proteins and plant bioactive compounds are sensitive to environmental conditions, improper processing may reduce vaccine efficacy and nutritional quality. Therefore, understanding the effects of thermal processing, storage and preservation technologies is essential for developing stable and effective edible vaccine products (Rai and Chaturvedi, 2023). Thermal processing is one of the most important factors affecting antigen and phytochemical stability in leafy vegetables. Many recombinant proteins are heat-sensitive and may undergo denaturation or structural degradation when exposed to high temperatures during cooking, drying, or industrial food processing. Denatured antigens may lose their immunogenic properties, resulting in reduced vaccine effectiveness (Liu *et al*., 2020). Consequently, leafy vegetables selected for edible vaccine production are often consumed raw or minimally processed to preserve antigen integrity. Lettuce and spinach are particularly suitable because they can be eaten fresh without extensive thermal treatment. Heat processing may also alter the concentration and bioavailability of phytochemicals. Certain vitamins and polyphenols are highly susceptible to thermal degradation, whereas others may become more bioavailable following moderate heating due to cell wall disruption. For example, carotenoids such as β-carotene and lutein may exhibit increased bioavailability after mild heat treatment, while vitamin C and some flavonoids are significantly reduced during cooking (Parvez *et al*., 2021). Glucosinolates present in cruciferous leafy vegetables may undergo enzymatic hydrolysis during thermal processing, influencing the formation of bioactive isothiocyanates. Therefore, optimizing processing conditions is critical for preserving both vaccine antigens and beneficial phytochemicals.

Non-thermal processing technologies have emerged as promising alternatives for maintaining antigen and phytochemical stability. Techniques such as freeze drying, cold plasma treatment, pulsed electric fields and high-pressure processing can preserve biological activity while minimizing thermal degradation (Barba *et al*., 2021). Freeze drying is particularly effective because it removes moisture under low-temperature conditions, thereby protecting recombinant proteins and sensitive phytochemicals from degradation. Freeze-dried edible vaccine products additionally exhibit improved shelf life and thermal stability during transportation and storage. Advances in food technology continue to improve processing and storage methods for edible vaccine products. Encapsulation, nanoformulation, antioxidant incorporation and smart packaging technologies offer promising strategies for enhancing antigen stability and preserving phytochemical functionality. These innovations are expected to significantly improve the commercial feasibility, shelf-life and therapeutic performance of leafy vegetable-based edible vaccines in future healthcare applications.

7. Applications of leafy vegetable-based edible vaccines

Leafy vegetable-based edible vaccines have emerged as promising tools for the prevention and control of infectious diseases because of their ability to stimulate mucosal and systemic immunity through oral administration. Advances in molecular farming and plant biotechnology have enabled the successful expression of several pathogen-derived antigens in leafy vegetables such as lettuce, spinach, kale and mustard greens. These plant-derived vaccines have demonstrated encouraging immunogenicity in preclinical and clinical studies against bacterial, viral and parasitic diseases (Streatfield *et al*., 2021). Their low production costs, ease of administration and improved accessibility make them particularly valuable for infectious disease management in developing regions and during global health emergencies. Hepatitis B is one of the most extensively studied diseases in edible vaccine research. Hepatitis B virus (HBV) causes severe liver disorders including cirrhosis and hepatocellular carcinoma, affecting millions of people worldwide. Recombinant hepatitis B surface antigen (HBsAg) has been successfully expressed in several leafy vegetables, particularly lettuce and spinach, using plant transformation technologies (Dobrica *et al*., 2022). Oral administration of transgenic lettuce expressing HBsAg has demonstrated the ability to induce both mucosal and systemic immune responses in experimental models. Plant-based hepatitis B vaccines offer significant advantages over conventional injectable vaccines because they eliminate the need for cold-chain transportation and trained medical personnel. Furthermore, the oral delivery of HBsAg through leafy vegetables may improve vaccination coverage among populations with limited healthcare access.

Cholera remains a major public health challenge in many low-income countries where sanitation and clean drinking water are inadequate. Cholera is caused by *Vibrio cholerae*, which colonizes the intestinal mucosa and produces enterotoxins leading to severe diarrhea and dehydration. Leafy vegetables engineered to express cholera toxin B subunit (CTB) antigens have shown promising immunogenicity in oral vaccine studies (Kim *et al*., 2020). CTB expressed in lettuce and spinach can effectively stimulate intestinal mucosal immunity and induce protective antibody responses against cholera infection. Since cholera primarily affects the gastrointestinal tract, edible oral vaccines are particularly suitable because they directly target mucosal immune tissues involved in pathogen defense. Influenza viruses continue to pose serious global health threats because of their rapid mutation rates and pandemic potential. Conventional influenza vaccines require annual reformulation and large-scale industrial production systems that may not always meet urgent global demands. Leafy vegetable-based edible vaccines expressing influenza hemagglutinin and neuraminidase antigens have demonstrated the ability to induce protective immune responses in animal models (Shoji *et al*., 2021).

Lettuce and spinach are particularly advantageous for influenza vaccine production because of their rapid biomass accumulation and compatibility with transient expression systems. Plant-based influenza vaccines may provide scalable and economical alternatives for rapid vaccine deployment during seasonal outbreaks and pandemics.

Norovirus is another important target for edible vaccine development because it is one of the leading causes of acute viral gastroenteritis worldwide. Noroviruses spread rapidly through contaminated food, water and person-to-person contact, causing significant outbreaks in both developed and developing countries. Several studies have successfully produced norovirus capsid proteins in transgenic lettuce and spinach plants capable of inducing mucosal immune responses following oral administration (Lico *et al*., 2020). The ability of edible vaccines to stimulate gut-associated lymphoid tissues is particularly advantageous for protection against enteric viruses such as norovirus. Beyond these diseases, leafy vegetable-based edible vaccines are being explored for numerous other infectious pathogens including rabies, tuberculosis, malaria, human papillomavirus and enterotoxigenic *Escherichia coli*. Advances in molecular farming continue to expand the range of vaccine targets and improve antigen expression efficiency in leafy vegetables. Consequently, edible vaccines are increasingly viewed as valuable next-generation tools for infectious disease prevention and global healthcare improvement.

8. Recent advances in biotechnology and genetic engineering

Recent advances in CRISPR/Cas genome editing technologies have revolutionized edible vaccine development by enabling precise genetic modifications in leafy vegetables. CRISPR/Cas systems allow targeted insertion, deletion, or modification of genes involved in recombinant protein production, antigen expression and metabolic regulation (Manghwar *et al*., 2020). Compared with conventional transformation methods, CRISPR/Cas technologies provide greater precision, efficiency and reduced off-target effects. In edible vaccine systems, CRISPR/Cas-based engineering has been employed to enhance antigen expression levels, improve protein stability and optimize metabolic pathways in leafy vegetables such as lettuce and spinach. Gene editing can also eliminate undesirable plant traits and improve biosafety by removing antibiotic resistance markers commonly used in transgenic systems (Bharat *et al*., 2022). Furthermore, CRISPR/Cas-mediated chloroplast engineering has enabled high-level accumulation of recombinant vaccine antigens while minimizing transgene escape through pollen transmission. The ability to rapidly engineer disease-resistant and stress-tolerant vaccine-producing plants further strengthens the potential of CRISPR/Cas technologies in molecular farming. As genome editing tools continue to evolve, they are expected to significantly improve the efficiency, safety and commercial feasibility of edible vaccine production systems.

Nanobiotechnology has emerged as a powerful interdisciplinary field contributing significantly to edible vaccine development. Nanotechnology-based delivery systems improve antigen stability, intestinal absorption and immune activation through the use of nanoparticles, nanoemulsions, liposomes and nanofibers (Nikaeen *et al*., 2021). These nanoscale carriers protect recombinant proteins from degradation caused by gastric acids, digestive enzymes and environmental stressors. Nanoparticle-based formulations additionally enhance targeted delivery of vaccine antigens to mucosal immune tissues and improve uptake by antigen-presenting cells. Biodegradable nanocarriers composed of chitosan, alginate and lipid nanoparticles have demonstrated promising results in oral vaccine delivery systems (Gharat *et al*., 2023). Nanotechnology may also enable controlled antigen release and co-delivery of adjuvants or immunomodulatory phytochemicals. The integration of nanobiotechnology with molecular farming enhances the therapeutic performance and commercial viability of leafy vegetable-based edible vaccines. Furthermore, nanotechnology-based biosensors and smart packaging systems contribute to improved quality control and stability monitoring during vaccine storage and transportation.

Artificial intelligence (AI) and omics technologies are increasingly transforming edible vaccine research by enabling data-driven optimization of vaccine design, plant engineering and immunological analysis. Omics approaches including genomics, transcriptomics, proteomics, metabolomics and immunomics provide comprehensive insights into plant metabolism, recombinant protein expression and host immune responses (Misra *et al*., 2022). These technologies help identify genes, pathways and metabolites associated with improved antigen production and vaccine efficacy. AI-based computational tools are being used to predict antigen structures, optimize codon usage, design synthetic promoters and analyze immune interactions. Machine learning algorithms can rapidly process large biological datasets to identify optimal transformation strategies and predict vaccine performance (Khalifa *et al*., 2023). AI-driven modeling also supports the development of personalized vaccines and precision immunization systems tailored to specific populations or pathogens.

9. Future perspectives

The future of leafy vegetable-based edible vaccines is highly promising due to continuous advancements in plant biotechnology, food technology, molecular farming and immunology. As global healthcare systems increasingly seek sustainable, affordable and accessible vaccination strategies, edible vaccines may emerge as transformative tools for disease prevention and health promotion. One of the most important future directions involves the development of personalized edible vaccines tailored to individual immune profiles, age groups, nutritional requirements and disease susceptibilities. Advances in genomics, immunoinformatics and artificial intelligence may enable the design of customized vaccine antigens and dosage systems capable of improving vaccine efficacy and minimizing adverse reactions. Personalized edible vaccines could be especially useful for immunocompromised individuals, elderly populations and patients requiring targeted immunotherapy. Another important area of future development is the creation of climate-resilient vaccine crops. Climate change poses serious threats to agricultural productivity through rising temperatures, drought stress, salinity, emerging pests and unpredictable weather patterns. Developing stress-tolerant leafy vegetables capable of maintaining stable antigen expression under adverse environmental conditions will be essential for ensuring reliable vaccine production. Modern genome-editing technologies such as CRISPR/Cas systems and advanced breeding approaches may help develop resilient vaccine-producing crops with enhanced tolerance to abiotic and biotic stresses. Such crops could support sustainable vaccine production in diverse agroecological regions and improve global healthcare preparedness during future pandemics and environmental crises. The integration of edible vaccine production with precision agriculture and food technology represents another major future opportunity. Controlled environment agriculture systems such as hydroponics, vertical farming and smart greenhouses can provide optimized growth conditions for consistent antigen production and improved biosafety. Precision farming technologies involving sensors, automation, artificial intelligence and data analytics may enhance crop monitoring, nutrient management and recombinant protein yield optimization. Simultaneously, advances in food technology will improve vaccine stabilization, shelf life, formulation and commercialization through innovative processing and packaging methods.

10. Conclusion

Leafy vegetable-based edible vaccines represent an innovative and sustainable approach to modern immunization and preventive healthcare. This review highlighted the fundamental concepts of edible vaccines, their mechanisms of immune activation and the importance of leafy vegetables as efficient platforms for recombinant antigen production. Leafy vegetables possess several advantageous characteristics including rapid biomass production, high protein expression capacity, ease of cultivation and rich phytochemical composition, making them highly suitable for oral vaccine delivery systems. The review also emphasized the significant role of food technology in improving vaccine stability, processing, storage, safety and commercialization potential. Phytochemicals naturally present in leafy vegetables provide additional antioxidant, anti-inflammatory, antimicrobial and immunomodulatory benefits that may enhance vaccine efficacy and overall human health. Furthermore, recent advances in biotechnology, genome editing, synthetic biology, nanotechnology and artificial intelligence have accelerated the development of efficient and scalable edible vaccine systems. Leafy vegetables are likely to play an increasingly important role in future vaccine technology because they combine nutritional value with therapeutic functionality. Their potential applications in infectious disease management, veterinary medicine, mass immunization and low-resource healthcare settings demonstrate their global significance. However, several challenges related to dosage consistency, regulatory approval, biosafety, public acceptance and commercialization still require careful attention.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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