International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

The Promising Potential of Plant-based Edible Vaccines: A Review

Maxyl Joshua Guillermo[1], Edelweise Genelsa[1], Lyna Guiam-an[1], Julienne Ladaga[1], Isabel Maceren[1], Zyarrah Zaida Pangolima[1], and Jacqueline Abiso-Padilla [2]

1,2 San Pedro College, 12 C. Guzman St., Davao City, 8000

A B S T R A C T

The emergence of edible vaccines is a new concept made through scientific advances by biotechnologists. Edible vaccines are subunit vaccines wherein plants are genetically engineered for inducing protection from diseases by producing certain pathogenic components into the plant. The recurrence of harmful diseases in different countries, especially in third-world countries, gave prominence to which the significance of developing and identifying prompt and immediate solutions is emphasized for it to be regulated. Thus, this review is done with the aim to introduce the prospects that come with plant-based edible vaccines as a worldwide immunization option from controlling and preventing pathogenic diseases, as well as to discuss the methods required for its production, mechanism of action, choice of host plant, and the limitations and challenges associated with edible vaccines.

Keywords:Plant-based Edible Vaccines, Edible Vaccines, Vaccines

1. Introduction

A vaccine is a biological substance that protects people against bacterial and viral infections. Vaccines, often known as immunizations, rely on our immune system's natural ability to protect us against infectious disease. The immune system is a machine that works around the clock to defend against invaders. Symptoms such soreness of the throat or high body temperature may be prolonged until the immune system is finally able to combat the pathogens. In order to guarantee a full reaction, more than one vaccine is usually required. Vaccines operate by inducing an antibody memory response in the body without producing sickness. One is able to build immunity without becoming ill when this happens [1].

For over two years now, the world has been facing COVID-19 pandemic. As of the moment, there are no specific treatments; rather healthcare providers rely on symptom management. As the years progressed, scientists have been conducting clinical trials for vaccine candidates. Currently, thirteen vaccinations have been authorized for use, and over ninety candidates are being tested in clinical studies [2]. Various vaccine categories are currently being studied and developed. These include: live, inactivated, subunit, vector, nucleic acid vaccines etc.

New vaccines were also not limited to the global pandemic. There are also emergence of new vaccines on diseases that are caused by arboviruses. Scientists see the potential of Insect-specific Viruses (ISV) which involves recombinant technology to generate chimeras [3]. Carvalho states that ISVs are safer since it does not replicate the vertebrate cells compared to attenuated vaccines and it does not need physical or chemical inactivation like inactivated vaccines. There is also an exploration on vaccines as prevention for antimicrobial resistance [4]. Furthermore, a promising novel system is emerging. Scientists eye for plant-based vaccines as an option for preventing coronaviruses [5].

Plant-based edible vaccines are subunit vaccines where a stable expression of an antigen is integrated into a plant's nucleus or chloroplast genome in a suitable vegetable or fruit such as banana, potato, etc[6]. This novel approach is currently explored as an option for preventing severe COVID-19 [5]. Unlike the commercial available vaccines, these oral vaccines are more cost-effective and economically efficient [7].

* *Maxyl Joshua Guillermo.* Tel.: +639453010373
E-mail address: *maxylj_guillermo@spcdavao.edu.ph*

Plant-based edible vaccines do not replicate human pathogens. It does need purification and it also eliminates the risk of contamination. Since it is an oral vaccine, usage of sterile injection is reduced and it does not require too much medical personnel for administration [6]. Therefore, it provides numerous advantages for mass immunization. With regard to this, the peer-review article aims to provide necessary information on a natural form of vaccine that is coming from edible plant sources as an alternative to synthetic vaccines.

2. Methods

This paper used different journals from online open-access databases and other online articles that offer information this study wishes to obtain. The topics searched were focused on plant-based edible vaccine -- its concept, mechanism of action, production, potential candidates, and limitations. All journals utilized in this peer-review article were adequately collected and selected to avoid any inconsistencies in information. Systematic review and synthesis were employed to ensure accuracy and coherence, thus a comprehensive peer-review article. Furthermore, the making of this paper started during the first week of November and ended in the last week of December.

3. Vaccines: Types and Sources

The development of vaccines enhances the immunity of humans by efficiently protecting people from harmful diseases and reducing the risks of acquiring the disease. Vaccines are made using different techniques such as by weakening the viruses (live, attenuated vaccines), inactivating viruses (killed or inactivated vaccines), inactivating toxins (toxoids), or by segmenting pathogens (subunit and conjugate vaccines). The live but weakened viruses used in the vaccines (attenuated vaccines) such as those made against mumps, varicella, influenza, rubella, and measles are recommended especially for the children in the United States [8].

As the majority of the vaccines are being administered through injection, the emergence of edible vaccines were fortunately made through scientific advances. This new concept of administering vaccines is an alternative way of delivering vaccines which involves incorporating a gene that is selected into the plant and the manufacture of protein that is encoded and is induced by the transgenic plant. Plants that can potentially be manufactured as edible vaccines are the banana, corn, rice, potato, lettuce, soybean and legumes can. This new concept vaccine administration is cost effective as it is also administered and stored easily. Also, edible vaccines are a widely acceptable type of delivery system to the patients of different ages. The development of edible vaccines, particularly in developing countries, is applicable to a vast array of diseases, namely: hepatitis B, diarrhea, measles, cholera, etc. [9].

Vaccines work by inducing an antibody memory response without really causing a sickness. A vaccine must have at least a single antigen from either bacteria or virus in order to trigger a response. Antigens can be employed in a variety of ways [10-11].

- Attenuated Live Vaccines - Attenuated live viruses are less potent variants of the original virus. They don't make you sick, but they do boost your immune system. Vaccines for measles, mumps, and rubella (MMR) and chickenpox are two examples.

- Inactivated Vaccines - Inactivated viruses are viruses that have been killed. In essence, antibodies are still produced despite the virus being inactivated. One such example is the polio immunization.

- Recombinant Vaccines - In creating this type of vaccine, genetic engineering is involved. Such a method can be used to copy a certain gene. The human papillomavirus comes in a variety of strains (HPV) and can be modified to prevent cervical cancer-causing strains.

- Conjugate Vaccines - This vaccine involves a covalent bonding between a weak antigen, usually a polysaccharide, and strong antigen, which is a protein. The Hib vaccine is an example.

- Subunit Vaccines - This component is the only one that uses antigens that cause an immune response. A subunit vaccine protects against influenza.

- Toxoid Vaccines - These vaccines utilize inactive forms of bacterial toxins to establish immunity. Vaccines against tetanus and diphtheria are two examples.

- mRNA Vaccines - In order to stimulate an immune response, mRNA vaccines produce proteins. When compared to other forms of vaccinations, mRNA vaccines have various advantages, including faster manufacturing times and no danger of disease in the person receiving the vaccine because they do not contain a live virus.

4. Concept of Plant-based Edible Vaccine

Plants have been prominent in pharmaceuticals since it has been a significant source of metabolites which are essential in the production of pharmaceutical products such as medicines, biologicals and such [12]. The biotechnology of plants has earned a gravity in this field as plant tissue culture has been useful in investigation of bioengineered plants and its products specifically in the creation of foods, medicines as well as plant-based biologicals. Plant tissue culture refers to techniques used to grow plant tissues in a nutrient media. This technique is essential in molecular farming since the stability of the expression largely depends on it [13].

Molecular farming, on the other hand, significantly helped the progress of subunit vaccine production [14]. Since traditional vaccines are expensive and complex, plants emerged as a bright prospect to become a system used to produce a broad-spectrum of active pharmaceutical protein that have a high value in the health industry. The advancement of biotechnology has led to transforming plants into potential bioreactors. Recently, plant-based novel systems have gained importance in the field where its trajectory points directly in developing edible or oral vaccines.

Edible vaccines come with numerous alternative names such as oral vaccines or green vaccines [15]. These are produced in an edible form regardless of its appearance and taste as long as it establishes safety in human consumption [16]. The idea of edible vaccines rests upon the conversion of consumable substances, specifically vegetables and fruits, into a potential vaccine in order to avert possible spread of communicable diseases [15]. Also, these forms of subunits are expressions of antigen to plant or animal-based products. In simple terms, edible vaccines are just made of plants or animal-based products which carry specific agents that will stimulate an animal's innate body defenses[17]. Since pathogens seize their hosts via mucosal surfaces such as the gastrointestinal surface, the creation of oral immunization makes a potential strategy for counteraction [18].

The first evidence of these vaccines was the expression of an antigen of *Streptococcus mutans*in tobacco [17]. The notion comes from the idea that since this bacterium causes dental caries, it is expected that once a mucosal immune response is stimulated to prevent this bacterium in colonizing the teeth it would consequently result in the avoidance of dental caries.

Therefore, these plant-derived edible subunits are one of the products of the advancement of biotechnology where any desired genes are introduced into plant tissues. These plant tissues will develop new encoded proteins that will be useful in countering pathogens. Compared to the traditional vaccines, it appears that plant-based edible vaccines are more cost-effective, safe, less invasive, tolerable, economic-wise and convenient [16].

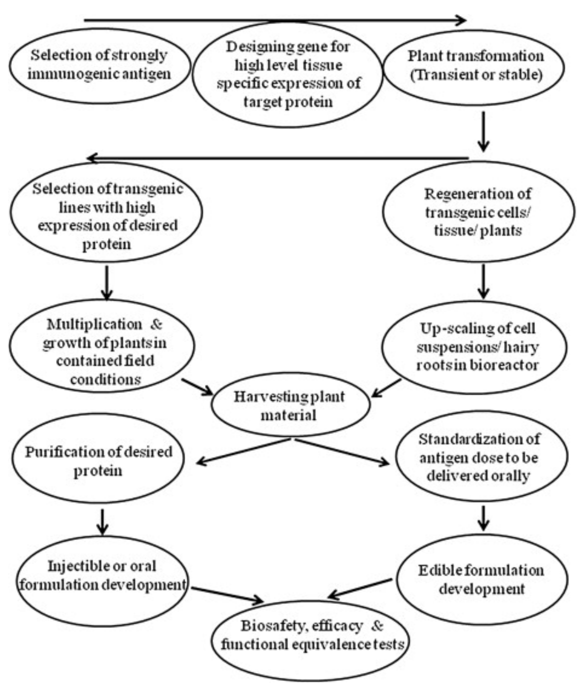

Fig. 1 - Diagram of Plant-based vaccines production (Adopted from Yadav D.K., Yadav N., and Khurana S.M.)[14]

5. Mechanism of Action

Plant-based edible vaccines are made to trigger the immune responses of the mucosa to combat foreign organisms called pathogens. Almost all human pathogens gain access to their hosts by invading the mucosal surfaces that line the digestive tract, respiratory tract, and reproductive tract, which comprise the largest immunologically active tissue found in the body [9]. Therefore, since oral vaccines can stimulate the body's first line of defense by producing antibody-mediated and cell-mediated immune responses and mucosal immunity, it is an effective method for mucosal immunization [19].

The following are three (3) elements of the immune system in the GIT that aid to better understand the mechanism of action of plant-based edible vaccines:

GALT (Gut-Associated Lymphoid Tissue)

It has the complex function of being the mucosal immunologic protectant of the GI tract against foreign disease-causing organisms [20]. It is made up of clustered tissues, such as clumped lymphoid nodules (or the Peyer's patches), isolated lymphoid sacs, as well as non-agglomerated cells located in the lamina propria, epithelial cells in the intestines or IECs, intraepithelial lymphocytes (IELs), and lymph nodes found in between the mesentery (MLNs) [21]. The GALT and the biggest lymph node in the body, the mesenteric lymph node (MLN), are key inductive sites of adaptive immune responses, while the lamina propria and mucosa epithelium have effector and memory roles [22]. As the main site of antigen exposure, the GALT conditions pre-mature T-lymphocytes as well as B-lymphocytes to mature into effector cells, which travels from the GIT to other parts of the body to guard against trespassing pathogens [21].

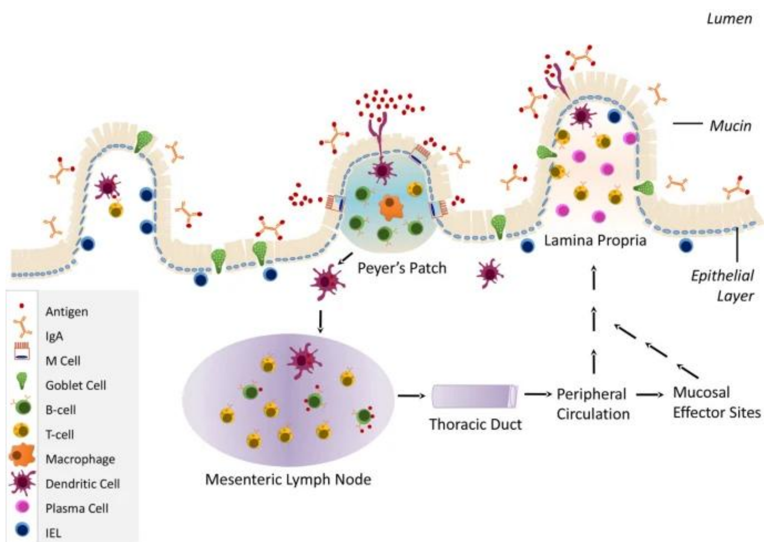

Fig. 2 - Illustration of GALT or Gut-Associated Lymphoid Tissue (Adopted from Ruth M.R. and Field C.J.) [21]

PPs (Peyer's Patches)

These are follicle clusters in the mucous membrane line the small intestine. These follicle clusters house various immunologic cells such as B lymphocytes, T lymphocytes, dendritic cells, and macrophages. More importantly, PPs are enclosed by the follicle-associated epithelium (FAE), which divides GALT and the microenvironment of the lumen. This cellular covering is densely packed with specialized epithelial cells called the microfold (M) cells [23].

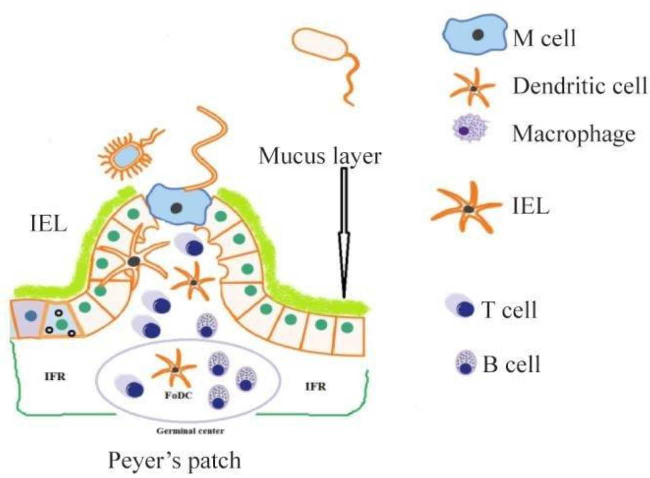

Fig. 3 - Illustration of Peyer's patch (Adopted fromAzizpour M., Hosseini S., andJafari P.) [24]

M cells (Microfold cells)

These are found in the epithelial tissues that cover the organized mucosal lymphoid tissues. These are specialized epithelial cells that act as a security system by sampling luminal microorganisms for mucosal immune monitoring [25]. Moreover, antigens are fed to the macrophages, dendritic cells, and B-lymphocytes in the Peyer's patches by these M cells through the process of transcytosis. Such a process activates immune responses that are antigen-specific, for instance the production of antigen-specific S-IgA. Antigen absorption mediated by M cells may aid in the maintenance of gut immune responses considering the importance of S-IgA in pathogen protection and the initiation of reciprocal connection with the gut microbial population [23].

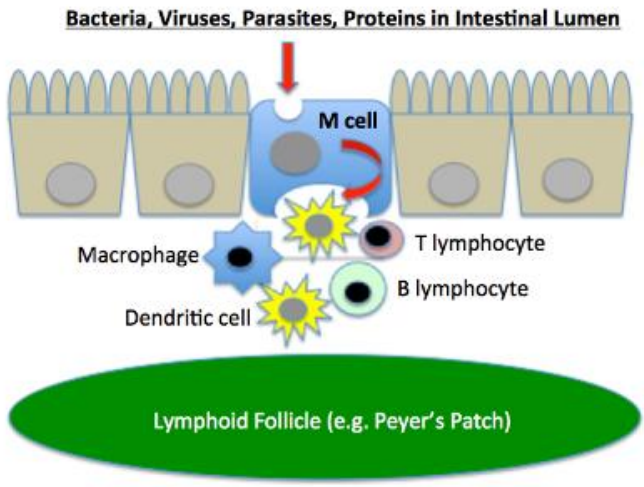

Fig. 4 - Illustration of Microfold cells (Adopted from Bowen R.) [26]

Plant-based edible vaccines act by prompting the mucosal immune response to release antibodies and neutralize any pathogens on sight. These edible vaccines have rigid outer walls that shield the antigens inside from enzymes and stomach fluids through a process called bioencapsulation[19]. Once it reaches the intestines, it will then get hydrolyzed and release its antigens. The released antigens are picked up by M cells. M cells have high transcytosis potential for various microorganisms and macromolecules [9]. The disintegration of edible vaccines happens close to the Payer's patches, which is made up of around 30 to 40 lymphoid nodules and comprises follicles from which the germinal center grows in response to antigenic stimulation [9]. When the antigen enters the epithelium of the gut through these follicles, it collects the antigen inside the lymphoid structure. Once inside, it will encounter M cells and be transported through the mucous membrane to the antigen-presenting cells like dendritic cells, where the breakdown of antigens happens through the process of endocytosis. The broken-down antigens will activate helper T-cells. These activated T-cells will produce cytokinins in the lymphoid follicles to attract B-cells [9]. After which, they will mature into plasma cells in the MALT (mucosa-associated lymphoid tissue). The now matured plasma cells will finally migrate to the mucosal membranes, where it will release immunoglobulin A (IgA) [9, 19]. The released IgA antibodies will be carried by epithelial cells into lumen secretions, where they attach to the antigens that are present in the lumen and neutralize it in an instant [27].

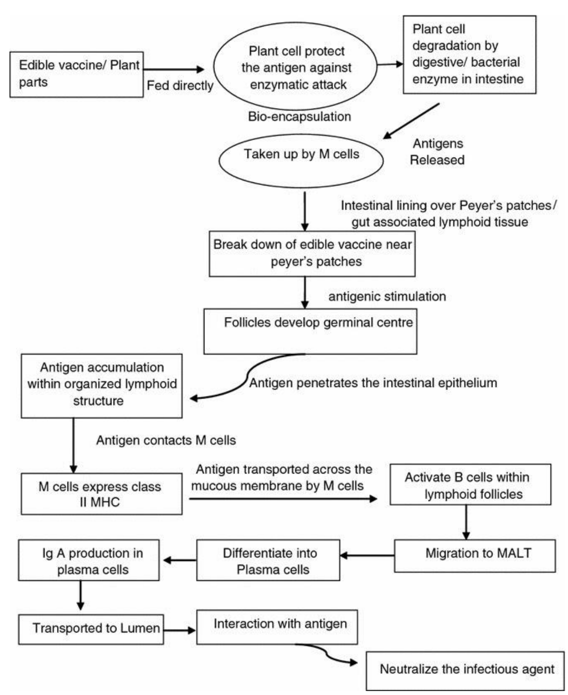

Fig. 5 - Diagram of the MOA of Plant-Based Edible Vaccines (Adopted from Saxena J. andRawat S.) [9]

6. Production of Edible Vaccines

6.1. Direct Gene Delivery Method

Among all the methods, the direct gene delivery method is the easiest because the selected DNA or RNA is directly introduced into the plant cell. The most utilized direct gene method is the ballistic method or also known as the gene gun or microprojectile bombardment method which is a vector-independent technique. This is utilized when quality trade or exchange is not possible [28]. In this method, the DNA or RNA is enfolded with Au (gold) or W (tungsten) which goes about as a microcarrier. So with the DNA or RNA enfolded, it will be set into the utilized kind of method which is the gene gun and introduce high tension of helium gas. The enfolded DNA will show action or basically it will move because of high strain or pressure and get penetrated into the designated plant cell. Also in the biolistic method there are two types of antigen expression that can be made, the nuclear transformation and chloroplast transformation. Nuclear transformation is the consolidating or combining of wanted genes into the core gene of the plant cell through homologous recombination, also the chloroplast transformation, the gene that is infused to the chloroplast to build protein articulation [29]. The upsides of chloroplast transformation contrasted with nuclear transformation is that the capacity for it to abolish the gene silencing effect, a fast and minimal expense creation because of its high duplicate in a plant cell, its capability to express various qualities in plastids and less specialized work, natural transgene regulation, and guaranteeing a site explicit insertion of the transgene in the chloroplast genome [30]. The method that is commonly used or taken for the making of edible vaccines is the chloroplast transformation. The only downside of this method is that it requires exceptionally significant expenses and may harm the plant [31].

6.2. Indirect Gene Delivery Method

This method can be over and done with combining transgenes into its designated plant cell. In spite of using the direct delivery method it shows critical adequacy or more significant efficacy in vaccine production as this method includes the use of plant microbes particularly the agrobacterium species of the plant which normally contaminate plant cells and can coordinate the quality of interest into the plant genome [32].

6.3. Agrobacterium-mediated Gene Transfer

This gene transfer is a gram negative bacteria which attacks the plant and transfers their genes to the plant nucleus. The commonly used biological vectors are the two strains of agrobacterium species which are the *Agrobacterium tumefaciens and Agrobacterium rhizogenes;* the difference between these two is the plasmid they convey [33]. *Agrobacterium tumefaciens*is the most preferred stain by researchers for steady articulation, which there are qualities encoding for plant chemicals. These genes are eliminated for vaccine production. This method is utilized to yield a stable integration of the antigen into the plant genome. In any case, it is straightforward and cost effective [34].

6.4. Genetically Engineered Plant Virus

Basically, in this method a suitable virus is reformed to a chimeric gene for viral coat protein. With this, it goes about as a vector to convey genetic materials into a cell [9]. This method shows transient articulation. The recombinant infection is a result of viral replication that states the ideal protein or peptide as the result. Furthermore, the synthesis and gathering of vaccine epitopes can be done by adjusting viral protein capsids. A few advantages of mediated infection includes disease incorporating a significant degree of recombinant protein expression inside a brief timeframe after infection, effectiveness to produce different antigen duplication the viral molecule surface [35]. Notwithstanding, the results from the replication must be sanitized first or have to be purified from the contaminated plants prior to being utilized for the vaccine. This technique will likewise be the result of death for plants after contamination [36]. Along these lines, when the vaccines have been gathered, another plant should be infected with recombinant virus and reinfection procedure must be done over and over again for ceaseless vaccine production.

7. Edible Vaccines Candidate/Choice of Host Plant

A desirable choice of a host plant that is both edible and palatable is necessary if it is intended for consuming raw. Edible vaccines are both applicable for animals and humans. However, the selection of plants for animal use should preferentially be selected according to the animal's diet [9].

Tobacco

The initial production of edible vaccines were successfully engineered on tobacco which is dated back in 1990 where it was found that there is 0.02% of recombinant protein of total soluble leaf in the gram-positive bacteria *Streptococcus*. Genetically modified tobacco is successful to be a host plant for the production of edible vaccines in combating the Hepatitis B antigen by using its gene to produce the vaccine. In addition, antibodies against dental caries and edible vaccines for HPV or human papillomavirus are currently in production which are expressed in the tobacco plant [9].

Potato

Potato plants are one of the first plants that are used in the synthesis of edible vaccines. One desirable option for plant-based vaccines are potatoes that are modified genetically. Potato-based vaccines have been successfully developed against Norovirus. This type of vaccine exhibited no adverse side effects in preclinical trials. Research is also done for transgenic potatoes which are engineered with cholera antigen and hepatitis B vaccine where both effectively boosted the immunity of the mice, in potatoes with cholera antigen, and first human trials, in potatoes with hepatitis B vaccine [9].

Tomato

Tomatoes are fine candidates for edible vaccines as they can be easily genetically manipulated and they grow quickly. The engineered tomatoes are either made as a paste or dried in aiding the delivery because the plant does not thrive well in areas where edible vaccines are mostly needed. This plant is used for HIV antigen which makes them an ideal candidate as compared to other transgenic plants, as this carries the protein, edible and immune to any thermal process, making this plant aid in retaining its healing capabilities. Tomatoes can also be used for the edible vaccine against Alzheimer's disease and rabies virus [9].

Banana

Bananas are widely available especially in third-world countries. It can be eaten raw by children, which is an advantage over other transgenic plants because this doesn't require cooking when consuming this. Banana-based vaccines are synthesized for HBV which is presumed to cost for only around 2 cents per dose, in comparison to injectable vaccines which are $125 [9].

Maize

People infected with hepatitis B who are at high-risk of liver diseases will receive the most benefit with maize or corn-based vaccines as this plant is used for the production of a protein HbsAg. Researchers aim for both pigs and humans who consume corn or corn products to get a flu vaccination. Birds can also be vaccinated or provide protection from diseases that are fatal and highly contagious which affects the majority of the bird species. It is believed that corn-made vaccines are effective for HIV vaccines where the corn is genetically modified. Transgenic corn is also produced for the rabies virus [9].

Rice

Rice grain, among other transgenic plants, are feasible as an oral immunotherapy agent for allergy treatment which contains T cell epitope peptides of allergens. The plant does not require refrigeration as it has a longer shelf life [9].

Spinach

Spinach, as a choice of host plant for anthrax vaccine and Human immunodeficiency virus type 1 Tat protein, is currently under investigation [9].

Furthermore, the study of Saxena and Rawat demonstrates in Table 1the advantages and disadvantages of the use of different plants as hosts for edible vaccines [9].

Table 1 - Features of different plant host systems (Adopted from Saxena J. and Rawat S.)[9]

Plant	**Advantages**	**Disadvantages**
Tobacco	• Transformation system is uncomplicated and efficient. • Protein characterization are obtained profusely.	• Oral administration is not compatible with toxic alkaloids. • Possibility of field outcrossing.
Banana	• Plant is widely available in third-world countries. • Can be eaten raw by both infants and adults • Clonal propagation. • Less probability for field outcrossing. • Affordable and abundant. • Accessible for a 10-12 month period.	• Transformation system is complicated • Limited gene expression data (fruit-specific promoters). • High demand for cultivation. • High greenhouse cost.
Potato	• Transformation system is uncomplicated and efficient. • Viable for raw consumption but not so palatable. • Fast assessment of the production of microtuber. • Clonal propagation. • Less possibility of field outcrossing. • Well-developed industrial tuber processing.	• Minimal tuber protein content. • Rancid when eaten raw. • Denaturation and decreased vaccine immunogenicity when cooked.
Tomato	• Less tuber protein content. • Can be eaten raw. • Accessible for fruit specific promoters. • Antigen gene stacking is achievable. • Industrial greenhouse culture and processing of industrial .fruit are well-	• Minimal fruit protein content. • Unsuitable for particular antigens or for infants due to the fruit's acidity. • No available techniques in vitro for fruit expression analyzation.

	developed.	
Legumes	• Widely utilized production technology. • High and stable protein content stored in seeds. • Suitable for animal vaccinations. • Well-developed industrial seed processing.	• Transformation system is complicated. • Denaturation and decreased vaccine immunogenicity when cooked. • Possibility of field outcrossing.
Alfalfa	• Uncomplicated and transformation system is efficient. • High protein content found on leaves. • Leaves can be eaten raw.	• Possibility of field outcrossing. • Field cleaning is difficult for deep root system.

8. Discussion

Since the 1990s, when its concept was first introduced, the effectiveness and safety of plant-based edible vaccines have been extensively investigated in the prevention of a variety of illnesses and infectious diseases [9]. In those years of study, plant antigen expression has been shown. The world's first plant-based edible vaccine clinical trial for humans used raw potatoes to provide immunity against a specific disease. Specifically, the subjects given transgenic potatoes that contained antigens against diarrhea, a condition caused by the bacterium *Escherichia coli*[17]. In the later years, further development and innovation in administering other antigens and other plants as edible vaccines have ensued.

Table 2–Current status of clinical trials (Adopted from Kurup V.M., Thomas J.) [38]

Pathogen	Antigen	Host	Disease	Clinical Trial Status
Rabies virus	Glycoprotein/nucleoprotein	Spinach	Rabies	Phase 1 (Early)
Norwalk virus	Coat protein	Potato	Diarrhea	
*Enterotoxigenic*E. coli	B subunit of heat-labile enterotoxin B	Potato/Maize	Diarrhea	
Hepatitis B virus	Surface antigen of Hepatitis B	Lettuce	Hepatitis B	
Hepatitis B virus	Surface antigen of Hepatitis B	Potato	Hepatitis B	Phase 1
Vibrio cholerae	B subunit of cholera toxin	Rice	Cholera	
Hepatitis B virus	Antigen of Hepatitis B virus	*S. cerevisiae*	Chronic hepatitis B	Phase 2
Hepatitis C virus	Hepatitis C virus antigen	*S. cerevisiae*	Chronic hepatitis C	

The use of edible vaccines has already been applied and tested to numerous health conditions and diseases.

a. Malaria

Development of plant-derived edible vaccines to combat malaria utilizes the following antigens: merozoite surface proteins 4 and 5 and merozoite surface proteins 4/5 from *P. falciparum* and *P. yoelii*, respectively [39]. Furthermore, in the study of Wang et al., oral immunization of mice with recombinant MSP 4, MSP 4/5, and MSPI as well as with cholera toxin B-subunit, when used as a mucosal adjuvant, elicited antibody responses that were effective against blood-stage infections [40].

b. Hepatitis B

According to the WHO, an estimated 296,000,000 individuals were infected with chronic hepatitis B in 2019, with 1,500,000 new cases per year [41]. Hepatitis B surface antigen (HbsAg) is currently utilized to produce the oral Hepatitis B vaccine. Moreover, clinical investigations using potatoes with the hepatitis B surface antigen (HbsAg) revealed that subjects who consumed these antigen-containing potatoes had higher antibody concentrations, demonstrating that edible vaccines are a feasible alternative to injectable vaccination [42].

c. Measles

An extremely contagious disease caused by Paramyxovirus. Recent findings have shown favorable results on the expression of hemagglutinin of the Paramyxovirus surface protein in lettuce, rice, potato, and tobacco. Additionally, the use of transgenic bananas in healthy experimental animals reported that the bananas could generate the antigenic hemagglutinin protein of the Paramyxovirus and trigger immunological responses [43].

d. Stopping Autoimmunity

Over the last decade, researchers have discovered multiple beta-cell proteins that can stimulate autoimmune responses in people suffering from Type 1 diabetes. In this process, tobacco and potato have been utilized to generate diabetic vaccines made from plants. The use of insulin or GAD coupled to benign *V. cholerae*toxin B-subunit was attempted in order to improve the antigen absorption of M cells. Results from feeding transgenic tobacco and potato to non-obese mice demonstrated an increase in the levels of IgG, an antibody linked to cytokines that reduce detrimental immune responses [42].

Edible vaccines offer a promising future for immunization. These vaccines promise a better prevention option from illnesses, more importantly in countries that have low-income. This is because it is cost-effective, efficient, and safe compared to traditional vaccines as agreed by several authors [9,16,38,44]. Reduced cost of production, purification, sterilization, distribution, and packaging in the long run is offered by the edible vaccines. Another factor for its cost-effectiveness is that it can be consumed raw, therefore reducing the cost for purification and processing and the potential degradation of the antigen is eliminated [16]. Its preservation also does not require the constant cold chains which leads to easier distribution [16, 38]. Additionally, the expression of antigens in seeds allows longer periods of maintenance and stability. Moreover, the need to transport and distribute will be eliminated if a local crop in a certain area is manipulated to yield the vaccine. Finally, the vaccine triggers defense at the mucosal surface that serves as the body's first-line of ammunition [16]. Among the journals reviewed for the advantages of edible vaccines, none have mentioned about the dosage of the vaccine.

9. Limitations and Challenges of Plant-based Edible Vaccines

Although the vaccine has great potential to offer better prevention from diseases, the production comes with challenges. Three journals [9, 16, 38] have mentioned about the inconsistency of the plant that can affect the strength of the medicine present in the food. These inconsistencies involve plant to plant variation such as their growth, development, type, and texture of its edible part. There are also food such as potatoes that require cooking which then may affect the strength of the medicine present.Administering edible vaccines to infants is inconvenient due to the possibility of them spitting it out [9]. Another limitation stated by several references [9, 16, 38] is the difference in dosage that results from the variance of edible material used. It was also mentioned that the evaluation of dosage is tedious and that there are uncertainties related to computation of dose that may necessitate several runs of medicament delivery [9, 16]. Additionally, dosage for children and adults are different. Another limitation related to dosage is the possibility of overconsumption of the vaccine which can be toxic and may lead to a concerning disease outbreaks [9], and may overstimulate the immune system [16]. A journal also stated that oral tolerance may be provoked by delivering the vaccine with too high a dose rather than counteracting bacteria or virus that assails the body [38].

Process of producing and distributing edible vaccines appears to be a limitation too. There are factors that contribute to the unguaranteed continuity of production. To begin with, the stability of vaccines in fruits is unknown and the selection of the best plant is difficult [9]. Following limitation is the necessity of assuring the grade and safety of the plants that will be used. Further, it is costly to store and maintain genetically engineered plants [16]. Continuity of production is also limited by the constantly changing nature of plants [38]. Moreover, protein constituents of the vaccine have the tendency to deteriorate due to the acidic pH of the stomach, and the presence of digestive enzymes that may affect the proteins [9]. Also, glycosylation processes in plants and in humans are different thus there is a possibility that the vaccine's functionality will be altered. And even if primary assessments of the vaccine have demonstrated favorable outcomes in humans, reaction towards the actual encounters with the virus is still unknown [38]. Studiesalso have mentioned the possibility of the plant-based edible vaccine to induce allergies and oral toleration during co-administration with oral additives [38, 44]. These reactions prove that the vaccine always comes with the possibility of side effects because of the interaction of the vehicle and the medicine [9]. The possible danger that comes with possible tainting of the oral conveyance system is also concerning. It is because of the cross-contamination that occurs between engineered and non-transgenic plants during plant fertilization. Sometimes, the DNA or the antigen may be released in bodies of water by means of insects and/or birds that came in contact with the modified plant which then causes water contamination, and may also accidentally enter the human cycle of food consumption which may enormously smite the populace of the fauna [44].

10. Conclusion

Edible vaccines have a big potential to change the entire situation about vaccines. Vaccines have a critical role in infection prevention, when looking back at the old times, vaccines were just limited to a minimum number of diseases but now not only human but also animal diseases of bacteria, microorganisms and even protozoan origin are being studied for vaccination. Not only this but the researchers are in progress to produce plant based vaccines for reaction disease or vaccines for autoimmune diseases like polygenic disease and cancer that has upped the requirement of those vaccines for developed countries conjointly which until recently were stressed to be necessarily majorly for developing countries. Except for several benefits of these vaccines, there are some relevant issues regarding them that are to be addressed. Besides, future analysis is required to overcome limitations like low expression, immune tolerance, glycosylation, immunogenicity, and stability of transproteins in the event that the reasonable application of those antibodies is to be figured out. One vital reason is the right coordination between exchanges to help these antibodies to contact individuals. Each technical and restrictive hurdle is out to overcome. It will be a challenge to form a positive public perception relating to safety and effectiveness of these vaccines after all the fuss created after all these years. In conclusion, funding for this analysis and participation of some big companies will definitely make this dream a reality soon.

Acknowledgements

The researchers would like to express their warmest gratitude to San Pedro College and their subject mentor, Mrs. Jacqueline Abiso-Padilla, MSPh, for making this opportunity possible. Their guidance and support have shed light through all the stages in writing this paper.

Disclosure of Conflict of Interest

No conflict of interest from the authors.

References

1. What are vaccines? [Internet]. Winchester Hospital. [cited 2021Dec2]. Available from: https://www.winchesterhospital.org/health-library/article?id=222982.
2. Yan Y, Pang Y, Lyu Z, Wang R, Wu X, You C, et al. The COVID-19 vaccines: Recent development, challenges and prospects. Vaccines [Internet]. 2021 [cited 2021Dec2];9(4):349. Available from: https://www.mdpi.com/2076-393X/9/4/349/htm
3. Carvalho VL, Long MT. Perspectives on new vaccines against arboviruses using insect-specific viruses as platforms. Vaccines [Internet]. 2021 [cited 2021Dec2];9(3):263. Available from: https://www.mdpi.com/2076-393X/9/3/263/htm
4. Vekemans J, Hasso-Agopsowicz M, Kang G, Hausdorff WP, Fiore A, Tayler E, et al. Leveraging vaccines to reduce antibiotic use and prevent antimicrobial resistance: A World Health Organization Action Framework. Clinical Infectious Diseases [Internet]. 2021 [cited 2021Dec2];73(4). Available from: https://academic.oup.com/cid/article/73/4/e1011/6119610?login=true
5. Rosales-Mendoza S, Márquez-Escobar VA, González-Ortega O, Nieto-Gómez R, Arévalo-Villalobos JI. What does plant-based vaccine technology offer to the fight against COVID-19? Vaccines [Internet]. 2020 [cited 2021Dec2];8(2):183. Available from: https://www.mdpi.com/2076-393X/8/2/183
6. Dutta J, Goswami S , MitraAbhijit, Chanda A, Chanda A. Environment-Friendly Plant-Based Edible Vaccines – A Novel Technique to Fight SARS-CoV-2 and Other Pandemics A Review. In: Multidimensional approaches to impacts of changing environment on human health [Internet]. 1st ed. Boca Raton, FL: CRC Press; 2022 [cited 2021Dec2]. p. 10. Available from: https://www.taylorfrancis.com/chapters/edit/10.1201/9781003095422-17/environment-friendly-plant-based-edible-vaccines-novel-technique-fight-sars-cov-2-pandemicsa-review-anurag-chanda
7. Brun A, Phan HT, Conrad U. Plant-Based Vaccine Antigen Production. In: Vaccine Technologies for veterinary viral diseases: Methods and protocols [Internet]. New York: Humana Press; 2016 [cited 2021Dec2]. Available from: https://link.springer.com/protocol/10.1007/978-1-4939-3008-1_3
8. Different types of vaccines [Internet]. History of Vaccines. [cited 2021Dec2]. Available from: https://www.historyofvaccines.org/content/articles/different-types-vaccines
9. Saxena J, Rawat S. Edible vaccines [Internet]. Ravi I, Baunthiyal M, Saxena J, editors. 2013 [cited 2021Dec2]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7120417/
10. Immunizations [Internet]. Vaccines and side effects: The facts. Healthy Children. American Academy of Pediatrics website; [cited 2021Dec2]. Available from: https://www.healthychildren.org/English/safety-prevention/immunizations/Pages/Vaccines-And-Side-Effects-The-Facts.aspx.
11. Immunizations in adults. [Internet]. Dynamed.com. EBSCO DynaMed Plus website; [cited 2021Dec2]. Available from: http://www.dynamed.com/topics/dmp~AN~T901365/Immunizations-in-adults.
12. Bhatia S. Application of Plant Biotechnology. Modern Applications of Plant Biotechnology in Pharmaceutical Sciences [Internet]. 2015 [cited 2021Dec2];:157–207. Available from: https://www.sciencedirect.com/science/article/pii/B9780128022214000054
13. Alireza T, Nader RE. Molecular farming in plants [Internet]. IntechOpen. IntechOpen; 2015 [cited 2021Dec2]. Available from: https://www.intechopen.com/chapters/48920
14. Yadav DK, Yadav N, Khurana SM. Vaccines: Present Status and Applications. Animal Biotechnology [Internet]. 2014;:491–508. Available from: https://www.sciencedirect.com/science/article/pii/B9780124160026000262
15. Saxena J, Rawat S. Edible vaccines. Advances in Biotechnology [Internet]. 2013 [cited 2021Nov18];:207–26. Available from: https://link.springer.com/chapter/10.1007%2F978-81-322-1554-7_12
16. Concha C, Cañas R, Macuer J, Torres M, Herrada A, Jamett F, et al. Disease prevention: An opportunity to expand edible plant-based vaccines? Vaccines [Internet]. 2017 [cited 2021Nov18];5(2):14. Available from: https://www.mdpi.com/2076-393X/5/2/14
17. Chaitanya VK, Kumar JU. Edible vaccines - sriramachandra university [Internet]. edible vaccines - Sri Ramachandra University. 2006 [cited 2021Dec2]. Available from: https://paperzz.com/doc/7977244/edible-vaccines---sri-ramachandra-university
18. KozakLjunggren M, Jedlina L, Basałaj K, Legocki A, Wedrychowicz H, Kesik-Brodacka M. A preliminary study of a lettuce-based edible vaccine expressing the cysteine proteinase of Fasciola hepatica for fasciolosis control in Livestock. Frontiers in Immunology [Internet]. 2018 [cited 2021Nov18];9. Available from: https://pubmed.ncbi.nlm.nih.gov/30483259/
19. Jan N, Shafi F, Hameed Obin, Muzaffar K, Dar SM, Majid I, et al. An overview on edible vaccines and immunization [Internet]. Austin Journal of Nutrition and Food Sciences. [cited 2021Nov29]. Available from: https://austinpublishinggroup.com/nutrition-food-sciences/fulltext/ajnfs-v4-id1078.php
20. Saavedra JM, Moore N. Microbiota of the intestine: Prebiotics [Internet]. Encyclopedia of Human Nutrition (Second Edition). Elsevier; 2006 [cited 2021Nov29]. Available from: https://www.sciencedirect.com/science/article/pii/B0122266943021906
21. Ruth MR, Field CJ. The immune modifying effects of amino acids on gut-associated lymphoid tissue [Internet]. Journal of Animal Science and Biotechnology. BioMed Central; 2013 [cited 2021Nov29]. Available from: https://jasbsci.biomedcentral.com/articles/10.1186/2049-1891-4-27
22. Wang X, Sherman A, Liao G, Leong KW, Daniell H, Terhorst C, et al. Mechanism of oral tolerance induction to therapeutic proteins [Internet]. Advanced drug delivery reviews. U.S. National Library of Medicine; 2013 [cited 2021Nov29]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3578149/
23. Kobayashi N, Takahashi D, Takano S, Kimura S, Hase K. The roles of Peyer's patches and microfold cells in the gut immune system: Relevance to autoimmune diseases [Internet]. Frontiers in immunology. Frontiers Media S.A.; 2019 [cited 2021Nov29]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6794464/
24. Azizpour M, Hosseini S, Jafari P. LactococcusLactis : A new strategy for vaccination [Internet]. [cited 2021Dec9]. Available from: https://www.researchgate.net/profile/Maryam-Azizpour-

2/publication/320149404_Lactococcus_lactis_A_New_Strategy_for_Vaccination/links/59e44cc20f7e9b97fbed6d78/Lactococcus-lactis-A-New-Strategy-for-Vaccination.pdf

25. Dillon A, Lo DD. M cells: Intelligent Engineering of Mucosal Immune Surveillance [Internet]. Frontiers. Frontiers; 1AD [cited 2021Nov29]. Available from: https://www.frontiersin.org/articles/10.3389/fimmu.2019.01499/full
26. Bowen R. M cells. [cited 2021Dec9]. Available from: http://www.vivo.colostate.edu/hbooks/pathphys/digestion/smallgut/mcells.html
27. Gunasekaran B, Gothandam KM. A review on edible vaccines and their prospects [Internet]. Brazilian Journal of Medical and Biological Research. AssociaçãoBrasileira de DivulgaçãoCientífica; 2020 [cited 2021Dec23]. Available from: https://www.scielo.br/j/bjmbr/a/ZwWDHygc479XjsgPN9PpZVm/?lang=en
28. Fauquet C, et al. Particle bombardment and the genetic enhancement of crops; Myths and realities. Molecular breeding. 2005;15(3):305-327 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7090473/
29. Chen Q, Lai H. Gene delivery into plant cells for recombinant protein production. Biomed Research International 2015. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7090473/
30. Gomez E. Developments in plant-based vaccines against diseases of concern in developing countries. The open infectious diseases journal. 2010;4(2):55-62. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7090473
31. Mason HS, et al. Expression of hepatitis B surface antigen in transgenic plants. Proceedings in the National Academy of Science USA. 1992;89:11745-11749. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7090473
32. Q. Chen and H. Lai, "Gene delivery into plant cells for recombinant protein production," BioMed Research International, vol. 2015, Article ID 932161, 10 pages, 2015. https://www.hindawi.com/journals/bmri/2015/932161/
33. Lee-Yoon Low, Shun-Kai Yang, De-Xian Andrew Kok, Janna Ong-Abdullah, Ngai-Paing Tan and Kok-Song Lai (September 19th 2018). Transgenic Plants: Gene Constructs, Vector and Transformation Method, New Visions in Plant Science, ÖzgeÇelik, IntechOpen, DOI: 10.5772/intechopen.79369. Available from: https://www.intechopen.com/chapters/63134
34. Bhattacharya A et al. The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Mol plant pathol. https://bioone.org/journals/the-arabidopsis-book/volume-2017/issue-15/tab.0186/Agrobacterium-Mediated-Plant-Transformation-Biology-and-Applications/10.1199/tab.0186.full
35. Fitchen JH, Beachy RN. Genetically engineered protection against viruses in transgenic plants. Annu Rev Microbiol. 1993;47:739-63. doi: 10.1146/annurev.mi.47.100193.003515. PMID: 8257114. https://pubmed.ncbi.nlm.nih.gov/8257114/
36. J. Yu and W. H. Langridge, "Novel approaches to oral vaccines: delivery of antigens by edible plants," Current Infectious Disease Reports, vol. 2, no. 1, pp. 73–77, 2000.https://link.springer.com/article/10.1007%2Fs11908-000-0091-z
37. Lal P, Ramachandran VG, Goyal R, Sharma R. Edible vaccines: Current status and future [Internet]. Indian Journal of Medical Microbiology. Elsevier; 2021 [cited 2021Nov29]. Available from: https://www.sciencedirect.com/science/article/pii/S0255085721021654
38. Kurup VM, Thomas J. Edible vaccines: Promises and challenges. MolBiotechnol. 2020;62(2):79–90. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7090473/
39. Parmar P. Edible vaccines: Applications, advantages and limitations [Internet]. BioTechnology Notes. 2016 [cited 2021Nov29]. Available from: https://www.biotechnologynotes.com/transgenic-plants/edible-vaccines-applications-advantages-and-limitations/627
40. Wang L, Kedzierski L, Wesselingh SL, Coppel RL. Oral immunization with a recombinant malaria protein induces conformational antibodies and protects mice against lethal malaria [Internet]. Infection and immunity. American Society for Microbiology; 2003 [cited 2021Nov29]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC153237/
41. World Health Organization. Hepatitis B [Internet]. World Health Organization; [cited 2021Nov29]. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b
42. Painter Z, Paules H, Schroth T. The development of an edible vaccine to prevent hepatitis b [Internet]. [cited 2021Nov29]. Available from: https://www.engineering.pitt.edu/contentassets/5a0c516991454a7482a98cd7a7ad5ada/papers/group-a-winners-2019/a3-che_best-session-paper_edible-vaccine.pdf
43. Tripurani SK, Reddy NS, Rao KRSS. Green Revolution vaccines, edible vaccines [Internet]. African Journal of Biotechnology. [cited 2021Nov29]. Available from: https://www.ajol.info/index.php/ajb/article/view/14901
44. V KC, JonnalaUjwal K. EDIBLE VACCINES [Internet]. Edu.in. 2006 [cited 2021 Nov 11]. Available from: https://www.sriramachandra.edu.in/university/pdf/research/journals/sep_2006/book_8.pdf