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Technological approaches for commercial production of functional food through fish farming: Opportunities and challenges

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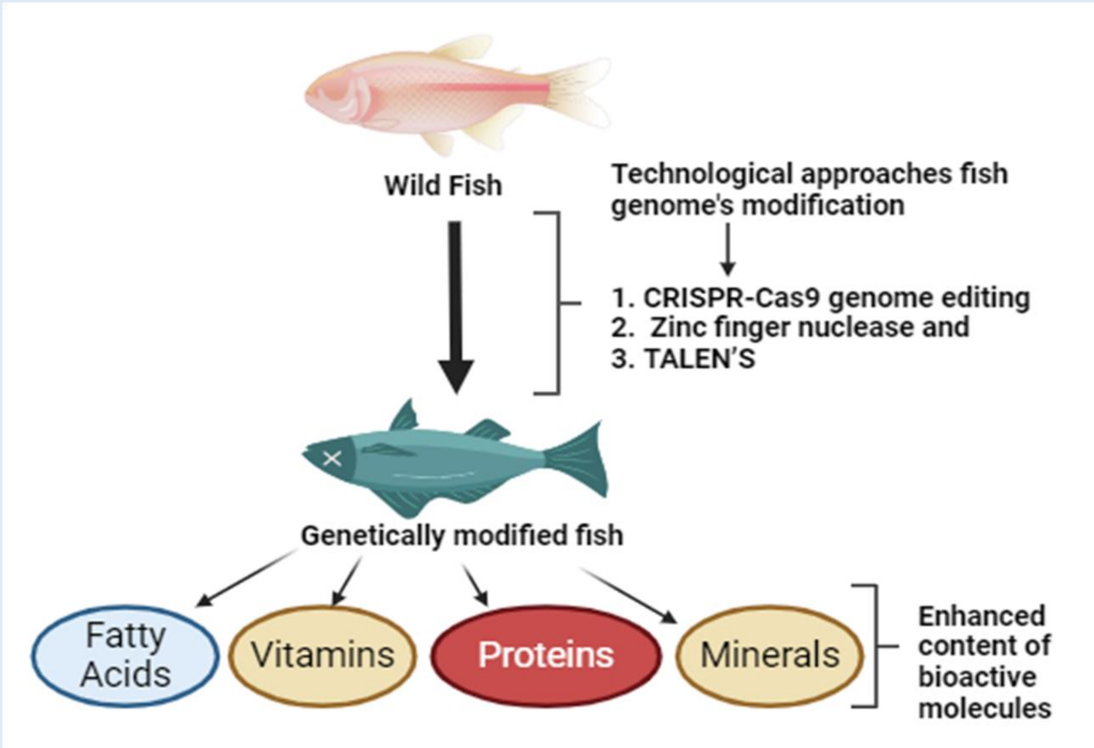
ABSTRACT

Functional foods and nutraceuticals have gained attention as a result of the emergence of several human health disorders, including abdominal, obesity, diabetes, cardiovascular diseases, and neurodegeneration. The components of functional foods influence the physiological system, which enhances human health and fights against illness. The global consumption of fish and fish products has increased in recent years, driven by the presence of omega-3 fatty acids, oligosaccharides, glucosamine, vitamins A, D, and E, as well as minerals such as magnesium, potassium, copper, sulfur, zinc, iodine, and proteins. It is widely acknowledged that functional food ingredients are important for promoting health, lowering the risk of disease, and lowering medical expenses. Fish farming has emerged as a source of novel functional ingredients from marine resources for functional food as well as for therapeutics. The review paper will also cover various technological approaches such as CRISPR-Cas9 mediated gene editing, zinc finger nucleases, and TALENs for enhanced production of bioactive compounds from genetically modified fishes for commercial production of functional food as well as nutraceuticals.

Keywords: Fish farming, Zinc finger nuclease, CRISPR/Cas9, Functional food, Nutraceuticals, Omega-3 fatty acids



Graphical abstract: Technological approaches for commercial production of functional food through fish farming: Opportunities and challenges

INTRODUCTION

Functional foods contain biologically active compounds or ingredients that provide health benefits beyond their nutritional content [1-2]. Functional foods are very effective, non-toxic, and help reduce the risk of infectious and chronic illnesses [3]. They reflect the traditional knowledge of communities that continue to use regional foods and traditional medicine [4]. Nutraceuticals have been shown to significantly reduce oxidative stress and enhance the antioxidant defense system in stressed individuals [5-7]. The increasing awareness among consumers about the nutritional value of food has encouraged the trend toward functional foods that offer medicinal benefits beyond basic nutrition [8]. Population growth has had a significant impact on food and therapeutic use, increasing the demand for food, which may lead to food insecurity, undernourishment, premature mortality, and susceptibility to chronic and infectious diseases. Environmental degradation further threatens food production and livestock. To address this challenge, alternative food resources that ensure sustainable food security and provide bioactive compounds with therapeutic applications, such as functional foods and nutraceuticals, are essential.

Aquafarming, also known as mariculture or aquaculture, is a large-scale production technology for aquatic organisms in controlled environments. It is used for seafood production, habitat restoration, and conservation breeding, involving organisms like algae, shellfish, and other marine organisms. Aquatic resources such as shellfish, marine pearls, seaweed, crustaceans, and molluscs serve as potential food sources. Fish farming, or pisciculture, is the most common form of aquafarming worldwide and could be a major sustainable food resource for the growing population. Fish farming provides nutritious food for a growing population, employment in rural areas, and additional income for farmers.

Fish and fish byproducts are rich sources of nutrients and proteins and have lower carbohydrate, cholesterol, purine, and saturated fatty acid content than other meats. Fish contains various bioactive compounds, including lipids, vitamins, and minerals, that have therapeutic effects, helping to prevent and manage conditions like cardiovascular disease, mental illness, hypertension, low birth weight, coronary diseases, eye diseases, dementia, and asthma.

Many fish species are used as food resources, including salmon, tuna, sardines, mahi-mahi, anchovy, herring, lingcod, moi, and orange roughy. Although these fishes contain bioactive compounds in lower concentrations, they hold potential pharmacological applications for acute and chronic diseases. Researchers are working on developing genetically modified fish using advanced recombinant technologies such as CRISPR-Cas9 mediated gene editing, zinc finger nuclease, and TALENs to increase therapeutic compound concentrations. This review aims to provide insights into fish as sustainable food resources, recombinant technologies for enhancing therapeutic production, and the challenges of genetically modified fish production

Classification of fish: Fishes can be classified based on their habitat as freshwater, globally utilized, and regionally utilized fish. Freshwater fish live in lakes, streams, and ponds, including trout, salmon, char (Salmonidae), carp, goldfish, minnows (Cyprinidae), perch, walleye, and darter (Percidae). Globally utilized fish, such as tilapia, carp, catfish, tuna, salmon, and Nile perch, are valued for their economic, cultural, ecological, and nutritional significance.

Fish farming: Fish farming, or pisciculture, involves the commercial rearing of fish in artificial environments like fish ponds or tanks, primarily for food. It includes various designs such as monoculture (single species), polyculture (multiple species), and monosex culture (only male or female fish). Common techniques include traditional freshwater pond systems, cage systems (enclosed in nets), integrated recycling systems (combining fish with hydroponic beds in greenhouses), and closed aquaculture systems, which are environmentally friendly. Extensive fish farming, using minimal external inputs like feed and additives, has been suggested as a scientific practice to enhance productivity [9].

Nutritional components of fish: Fish is considered one of Asia's most valuable commercialized foods due to its nutritional benefits. Enriched with vitamins, proteins, minerals, and polyunsaturated fatty acids (PUFAs), fish are critical species in marine ecosystems and one of the healthiest dietary options [10-11]. Several growth factors such as age, sex, feeding habits, temperature, adaptations, and hatching influence fish's nutritional composition of fish [11].

Fish provide high-quality proteins, vitamins (A, D, E, K, and most of the vitamin B complex), and minerals (phosphorus, iodine, calcium, iron, zinc, iodine, and selenium). Fish are also enriched in polyunsaturated fatty acids (PUFAs) (Figure 1) [12-13]. Studies have shown that some fish, consumed whole, offer ample calcium. Small fish like *Amblypharyngodon mola* are particularly enriched in vitamin A (retinol and dihydroretinol) [14].

Furthermore, fish contain significant nutraceutical compounds such as polysaccharides (glycosaminoglycan, chitin, and chitosan), lipids, minerals, and hydrocarbons like squalene and carotenoids, which are used in food supplements and medicine [15]. According to FAO, fish's muscle predominantly contains water (59.1-87.8%), lipids (0.8-23.5%), proteins (9-24%), and ash (1.6-6.2%). The detailed description of fish's bioactive constituents with their therapeutic potentials is explained as follows:

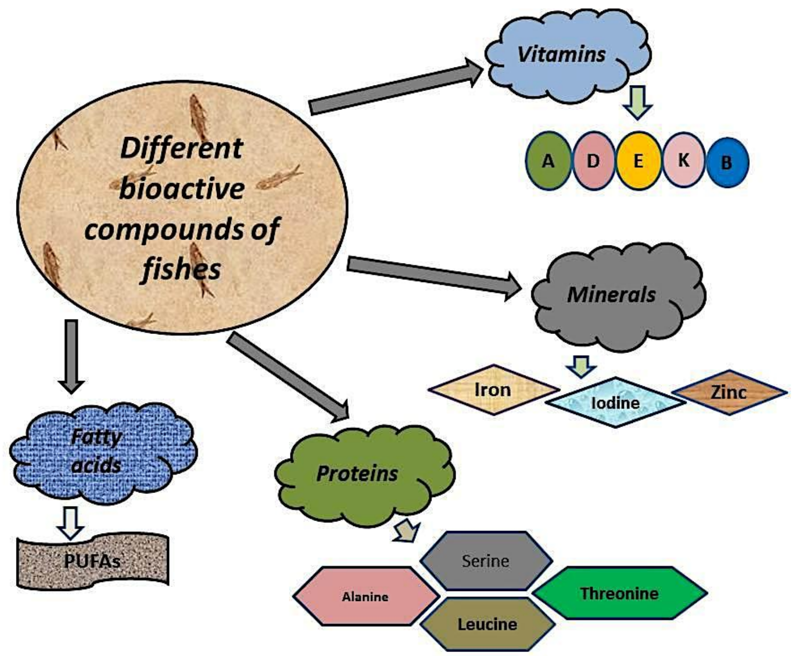


Figure 1. Bioactive compounds present in fishes

Protein: Proteins are highly complex macromolecules and biomolecules composed of one or more long polymers of amino acids. In simpler terms, proteins are made up of amino acids and have a bipolymeric structure, consisting of both essential as well as non-essential amino acids in biological systems. Approximately 9-24% of proteins are obtained from fish and fish products, as they contain a balanced composition of essential and non-essential amino acids composition [16]. Essential amino acids include tyrosine, histidine, phenylalanine, leucine, valine, isoleucine, methionine, threonine, tryptophan, and lysine, while non-essential amino acids include aspartic acid, glutamic acid, alanine, proline, serine, and cysteine.

All essential amino acids, including sulfur-containing amino acids like methionine and cysteine (which are absent in plant proteins), play a critical role in human nutrition [17]. Functional amino acids regulate key metabolic pathways, supporting growth, reproduction, and development [18]. These amino acids have potency to prevent and treat various metabolic issues like obesity, coronary heart disease (CHD), neurological disorder, type 2 diabetes, intrauterine growth restriction, infertility, and infectious disease [19-20].

Fish proteins, especially those acquired from lean fish, have been shown to reduce the risk of type 2 diabetes. In marine fish, "collagen" is a principal protein in connective tissue such as bones, scales, and skin. Marine collagen has various therapeutic applications in pharmaceuticals, nutraceuticals, and cosmetics due to its tissue regeneration, anti-aging, and bone repair [21]. The amino acids used as therapeutics are shown in Table 1.

Table 1. Amino acids used in disease treatment

Amino acids	**Fish species**	**Diseases**	**Reference**
Arginine	*Tor putitora* *Oncorhynchusmykiss*	Sepsis, hypertension, Preeclampsia	[22]
Glycine	*Catla catla* *Labeorohita* *Catla mrigala*	Anti-oxidant activity, diabetes, cardiovascular disease	[23]
Threonine	*Stolephoruswaitei* *Nemipterus japonicas*	Multple sclerosis Amyotrophic lateral sclerosis	[24]

Fatty acids: Fatty acids are considered the main component of lipids, which can be acquired from diets and exist as triglycerides, phospholipids, and cholesteryl esters. Fatty acids are made up of carboxyl and methyl groups on the opposite sides. Fatty acids are categorized into 2 subcategories: saturated fatty acids and unsaturated fatty acids. These fatty acids are major constituents of signaling molecules, hormones, and cell membranes and are considered energy sources. Saturated fatty acid (SFA) has no double bond, whereas unsaturated fatty acid may have one or more double bonds. Fatty acids are stored in the form of fats in the liver, muscles, and connective tissues [25]. Monounsaturated fatty acid (MUFA) involves a single double bond, and polyunsaturated fatty acid (PUFA) comprises two or more double bonds. PUFA is comprised of omega-3 like Alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and omega-6 like arachidonic acid (AA), Linoleic acid (LA), gamma-linolenic acid (GLA) fatty acids which are not synthesized by our body and hence it can be obtained through diet. The fishes that have high EPA and DHA are *Clarias gariepinus, Oreochromis niloticus, and Tor soro.* PUFA, MUFA and SFA are the principal key components of fatty acids. In children, docosahexaenoic acid (DHA) plays a major role in brain expansion. alpha-linolenic acid (ALA) is a prototype or precursor of DHA [26]. It has been reported that daily consumption of 250 g of eicosapentaenoic acid (EPA) and docosahexaenoic acid prevents chronic heart disease (CHD), whereas 150 mg/day intake improves brain development. Long-chain PUFA plays a pivotal role during lactation and pregnancy as mother provide long-chain n-3 PUFA to their neonates, which are responsible for the maturation of the central nervous system [27]. These fatty acids are responsible for the reduction in plasma triacylglycerol levels, preventing blood aggregation and blood pressure, and decreasing inflammation. Fish oil enriched in PUFA is efficient in curing various disorders like cancer, diabetes mellitus, Parkinson's disease, asthma, ulcerative colitis, etc. [28]. Omega-3 fatty acids have properties to activate cell signalling pathways and show neuroprotective properties [29]. Omega-3 fatty acids are crucial for the development of the brain and eyes, especially docosahexaenoic acid (DHA) [30].

Vitamins: Fishes are highly enriched in fat and water-soluble vitamins like A, B, D, E, K, and many more, which play a significant role in several processes such as vision, bone mineralization, and immune function [31]. Vitamins A, D, and E are most abundant in fish oils and fish species like Sardines, Mackerel, Herring, Lake trout, and Salmon. Vitamin A is very efficient in mitigating the risk of breast cancer, lung cancer, and colorectal cancer [32]. Vitamin E is responsible for the suppression of various signaling pathways that induce cancer progression, including 5 lipoxygenase, eicosanoids, and cyclooxygenase (COX) pathways. Vitamin D is isolated from Mackerel, Herring, Shrimp, and Marine trout and used in the treatment of cancer, asthma, and multiple sclerosis, whereas Vitamin A from Halibut and Cod Sardine fish is widely used in the treatment of xerophthalmia.

Minerals: Fishes are the preeminent source of crucial minerals and trace elements. That is why seafood is considered the most abundant amount of minerals compared to land-derived foods. Potassium, iron, selenium, calcium, and iodine are found in fish. Fish, exclusively its bone parts, is one of the reservoirs of calcium. Calcium plays a pivotal role in bone formation and growth of dental tissue particularly for children and pregnant women. Additionally, potassium and sodium aid in the transmission of nerve impulses and managing electrolyte balance. Iron is an ingredient of hemoglobin, which facilitates oxygen transport throughout the body [33]. Zinc functions as a cofactor and is crucial for various physiological processes such as digestion, nerve function, protein synthesis, immune cell development, and cell division [34]. Fishes and other marine food are regarded as natural sources of a nutrient called iodine. When a sufficient amount of iodine is not present in our body, it can lead to various problems, including cretinism, mental retardation, and other risk, especially in pregnancy.

Strategies used in fish modification: Different strategies have been utilized to alter the genome of fish. Interestingly, it is noted that genetically modified fish have greater advantages, including resistance to pathogen infection, rich nutritional quality, and enhanced growth rate. Genetically engineered fishes have been currently utilized in many disciplines of science, including pharmacology, experimental models, and medical science. *Glofish* was the first genetically modified fish. Various advanced techniques such as CRISPR/Cas9, ZFN, and TALENs are widely used in the modification of fish genomes. The characteristics of different genome editing tools are given in Table 2.

Table 2. Comparison of three prominent gene editing techniques (CRISPR-Cas9, ZFN, and TALENs)

Characteristics	**Clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9)**	**Zinc finger nucleases (ZFN)**	**Transcription activator-like effector nuclease (TALENs)**
Configuration	Guide RNA and Cas 9 endonuclease	Zinc finger DNA binding domain hybridizes with FOK 1 endonuclease	TALENs DNA hybridizes with FOK 1 endonuclease
Recognition site diameter	23 DNA bases	9-18 bases	30-36 bases
Design feasibility /simplicity of design	Simple as compared to ZFN and TALENs	More complex than TALENs and CRISPR	Simple as compared to ZFN
Potency	Efficient	Highly efficient	Efficient
Expensive	More expensive	Less expensive	More expensive

CRISPR-Cas9 mediated genome editing: In the discipline of biotechnology, CRISPR-Cas9 is among the most widely used and advanced genetic engineering tools. It is an emerging technique in biotechnology for genome editing and has significant benefits like easier, more efficient, and lower cost than ZFN and TALENs (Table 2). In this technique, RNA-guided endonucleases can perform genome editing, which enables the appropriate modification of genes. CRISPR-Cas9 consists of two necessary constituents: a Cas9 endonuclease and single guide RNA (sgRNA), which are associated with tans activating CRISPR RNA (traCrRNA) and CRISPR RNA (crRNA). Genome editing in fish via CRISPR-Cas9 technology is summarized in Table 3.

Table 3. Editing the genomes of multiple fish using CRISPR-Cas9 technology.

Fish species	**Target gene modification**	**Modified trait**	**Reference**
Channel fish *Ictaluruspunctalus*	Myostatin (mstn)	Growth	[35]
Tiger puffer *Takifugurubripe*	Leptin receptor	Growth	[36]
Nile tilapia *Orechromis niloticus*	Slc 45 a2	Pigmentation	[37]
Atlantic salmon *Salmosalar*	Tyrosinase (tyr)	Pigmentation	[38]

Zinc finger nuclease (ZFN): Zinc finger nuclease is one of the most advanced emerging genetic engineering techniques that utilize nucleases for editing genes in different species with precisely intentional changes [39]. Zinc finger comprised of two cysteine and histidine tandem arrays of zinc finger. The DNA-binding as well as cleaving domains are the two subunits that form active zinc finger nuclease. DNA-binding domains bind at the target site, whereas DNA-cleaving domains identify and cut DNA at specific sites [40]. ZFN are designed in pairs that can be separately worked for each DNA strand and responsible for inducing double-stranded breaks in pre-determined DNA, and it could be restored by a non-homologous end joining (NHEJ) DNA repair mechanism (Figure 2).

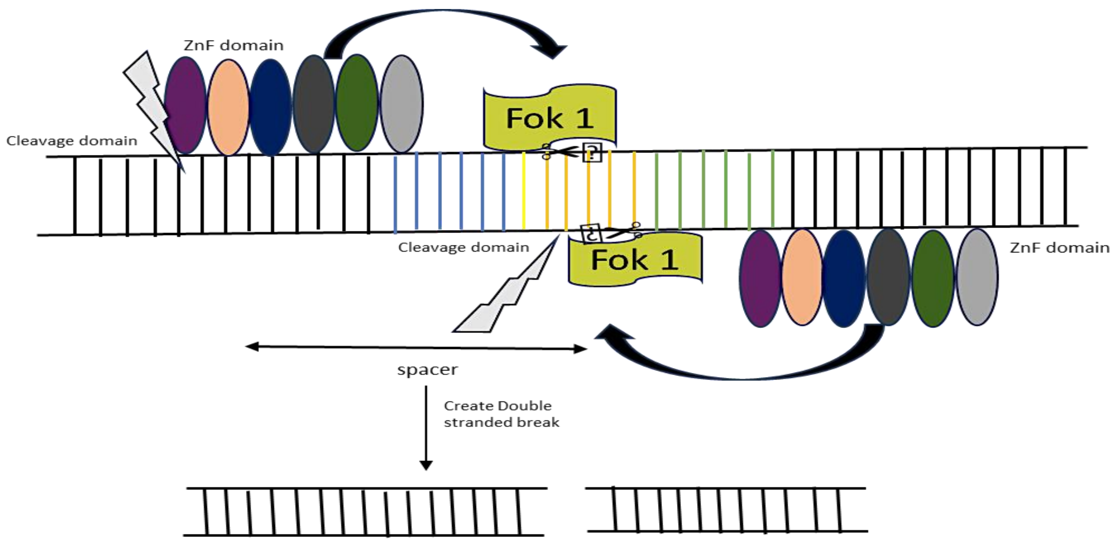


Figure 2. Zinc finger nuclease-mediated genome modification

Transcription activator-like effector nuclease (TALENs): TALENs are an advanced technique for genome editing and are associated with specialized effectors protein, comprised of FOK 1 nuclease subunit and DNA binding domain [41]. TALENs are responsible for triggering double-stranded breaks, and their domains are always worked in duplexes as dimers. TALENs are employed as one of the advanced emerging tools for genome editing in the field of biotechnology, as they target specific sites of genes of interest. Nuclease involved in TALENs has a specific central domain for binding of DNA and specialized or unique N and C terminus for localization and activation [42]. One nucleotide of the target DNA sequence binds with the DNA-binding domain, which consists of 10 to 30 monomer repeats. Additionally, TALENs consist of a non-specific target site present in the nucleus, which is associated with the FOK 1 domain and positioned at the C terminus and cleavage happening at the "SPACER" sequence [43]. As with ZFN, double-stranded breaks in TALENs are also repaired by the non-homologous end joining (NHEJ) method (Figure 3). Xiang *et al.*[44] reported that genome editing in Zebrafish was carried out by using TALENs technique.

Future perspectives and challenges: Fish farming is one of the essential factors in the food sector. Fishes could be used in disease treatment, especially in regenerative medicine and drug discovery [45]. Bioactive constituents, including lipids, proteins, fatty acids, and minerals, offer potential roles in disease treatment [46-47]. One of the major challenges is the isolation of bioactive compounds from fish. This process is cumbersome and time-consuming. The extraction method is very challenging, and it is necessary to evaluate the safety and toxicity of bioactive constituents from fish for their nutraceutical application [48]. Fish bioactive compounds are used in various disease treatments like cancer, diabetes, neurodegenerative disease (PD, AD), and obesity [49]. Fish-derived bioactive constituents like omega-3 fatty acids are used in CVD, reducing inflammation, and suppressing triglyceride levels as well as other constituents. However, these compounds do not completely cure the disease. Lactobacillus containing industrial products has potential bioactive compounds that provide many health benefits and give an idea for the possible use of fish and its products in future research [50]. Future research is ongoing to explore the pharmacological properties of fish bioactive constituents, which hold promising opportunities in medical attributes. Researchers are interested in identifying novel bioactive compounds derived from fish that have the potential for disease treatment. Additionally, advancements in biotechnology might influence the synthesis of these compounds on a large scale through advanced genetic engineering tools. Aquaculture and pharmaceutical industries work synergistically, resulting in advancements in innovative therapies that may be utilized for various health problems. Fishes or fish products from marine systems provide safe, green, and nutritious raw materials for the commercial production of functional foods, which promotes the development of the functional food industry [51-52]. However, genetically modified fishes have an ethical issue potentially raised by the use of GM technologies in aquaculture.

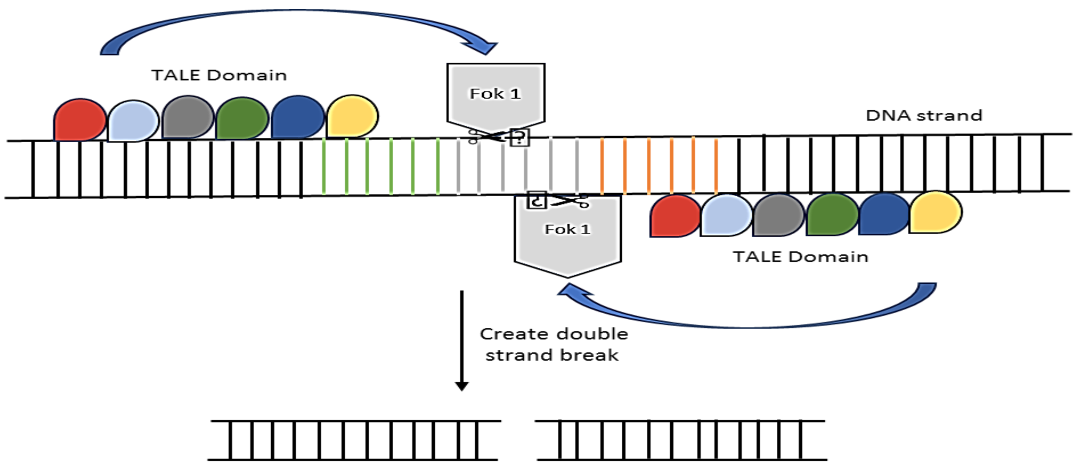


Figure 3. TALENs-mediated genome modification

CONCLUSIONS

Various innovative methods have been implemented into the advancement of aquaculture which is really very efficient and environmentally friendly. Fish farming presents a significant advancement as it can raise lots of fish cultivated with biological adaptation to flourish in different environments. Farmers are economically benefitted from fish farming as they can grow lots of fish. Zebrafish is one of the crucial model organisms for genetic modification. The findings of this review showed that fish could be a good source of food resources for a growing population, and modified fishes produce high quality and quantity of bioactive compounds like lipids, proteins, fatty acids, and minerals that have medicinal applications.

List of abbreviations: AA: Arachidonic acid, AD: Alzheimer disease, ALA: Alpha-linolenic acid, CAS-9: CRISPR-associated protein 9, CHD: Coronary heart disease, COX: Cyclooxygenase, CRISPR: Clustered regularly interspaced short palindromic repeats, crRNA: CRISPR RNA, CVD: Cardiovascular disease, DHA: Docosahexanoic acid, EPA: Eicosapentaenoic acid, GLA: Gamma linolenic acid, LA: Linoleic acid, MUFA: Monounsaturated fatty acid, NHEJ: Non-homologous end joining, PD: Parkinson disease, PUFAs: Polyunsaturated fatty acids, sgRNA: Single guide RNA, TALEN'S: Transcription activator-like effector nuclease, traCrRNA: tans activating CRISPR RNA, ZFN: Zinc finger nuclease.

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