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Review article

Repurposing chitin-rich seafood waste for warm-water fish farming

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ABSTRACT

The pisciculture industry has grown multi-fold over the past few decades. However, a surge in development and nutrient demand has led to the establishment of numerous challenges. Being a potential solution, chitosan has gained attention as a bio nanocomposite for its well-acclaimed properties including biodegradability, non-toxicity, immunomodulatory effects, antimicrobial activity, and biocompatibility. This biopolymer and its derivatives can be transformed into various structures, like micro and nanoparticles, for various purposes. Consequently, with regards to these properties chitin and its derivatives extend their application into drug delivery, food supplementation, vaccination, and preservation. This review focuses on the clinical advancements made in fish biotechnology via chitosan and its derivatives and highlights its prospective expansion into the pisciculture industry—in particular, warm-water species.

1. Introduction

Seafood has been a part of the human diet since ancient times. So much so that early cave drawings illustrate fishing and consumption of seafood. However, due to overfishing and excessive wastage of seafood in recent years, there has been a global economic loss and decline in fishes and crustaceans, which has caused immense damage to the ecosystem. The USDA defines food waste as “the component of food loss that occurs when an edible item goes unconsumed, as in food discarded by retailers due to colour or appearance and plate waste by consumers,” [1].

Despite significant advancements in processing, refrigeration, and shipping, millions of tonnes of aquatic products are wasted or have their nutritional value diminished annually. In fisheries and aquaculture, it is estimated that 35% of the global harvest is either lost or wasted every year [2]. The United Nations under the Sustainable Development Goal (SDG) has laid down to reduce the waste by

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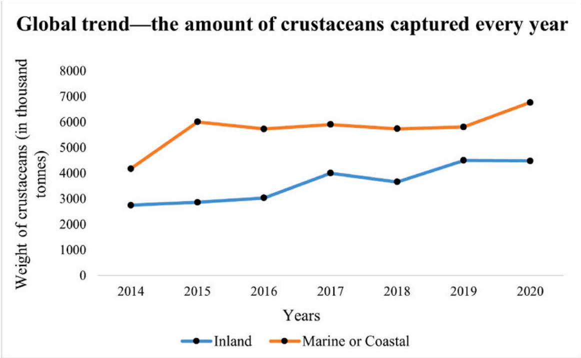

Fig. 1. The trend shown in the graph brings focus to the potential and available opportunity for extracting chitin from inland and marine crustaceans. An approximately increasing trend from 2500 to 3500 tonnes of inland captures between 2014–2020 and 4000–7000 tonnes of marine captures are highlighted [2,3,7–11].

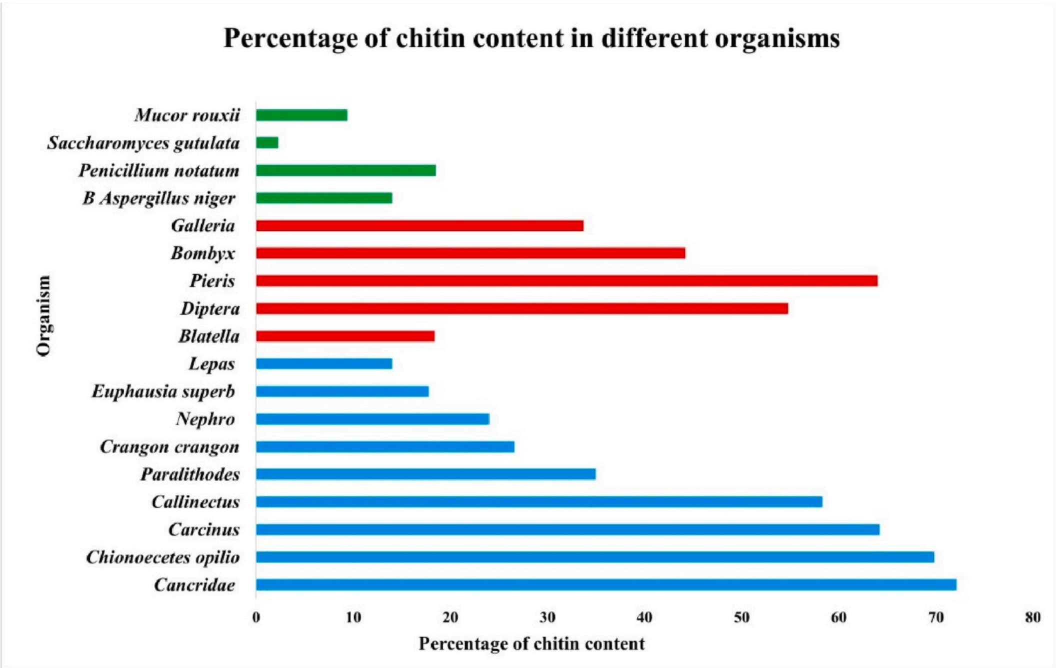

Fig. 2. Figure representing the percentage of chitin content in crustaceans (blue), insects (red) and fungi (green)—(blue) based on the weight of the organic cuticle; (red) dry weight of the body; (green) dry weight of the cell [12–15]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

half by 2030 to create a world with zero hunger, poverty and improved well-being [3].

Annually, living species in the ocean create 10^{12}–10^{14} tonnes of chitin, of which 1.3×10^{12} tons is produced by marine animals, and 2.8×10^{10} tons is produced by freshwater arthropods [4]. The seafood sector produces roughly 106 tonnes of waste annually, the majority of which is composted or used to make low-value items like animal feed and fertilizer. Furthermore, approximately 2000 tonnes of chitosan is generated each year, most of which comes from leftover shrimp and crab shells [5]. The seafood traded annually generate 6 to 8 million tonnes of trash crab, shrimp, and lobster shells on an average [6]. The amount of the crustacean captured every two years is illustrated in Fig. 1.

Crustacean shells constitute 20–40% protein, 20–50% calcium carbonate and 15–40% chitin [12]. Fig. 2 represents the chitin percentage in different organisms, and elucidates that chitin is predominantly found in crustacean shells at a higher percentage.

The Chitin market was estimated at $42.29 billion in 2020 and is anticipated to increase at a compound annual growth rate (CAGR) of 5.07% from 2021 to 2028 to reach $69.297 billion [16]. In 2019, Asia Pacific emerged as the largest geographic market. As a result of the quick expansion of end-use industries in Japan, China, India, and South Korea, it is also anticipated to have the fastest growth rate. This surge in demand can be attributed to the region's demand for chitosan as a biobased good and a conducive economic climate

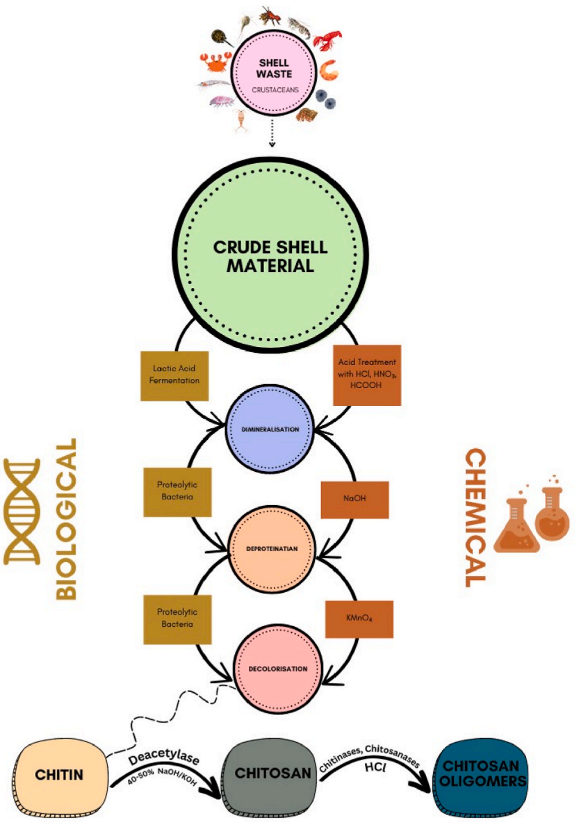

Fig. 3. An overview of steps involved in obtaining chitosan and its derivatives from crustacean shell waste. Adapted from Junceda-Mena et al., 2023 [18].

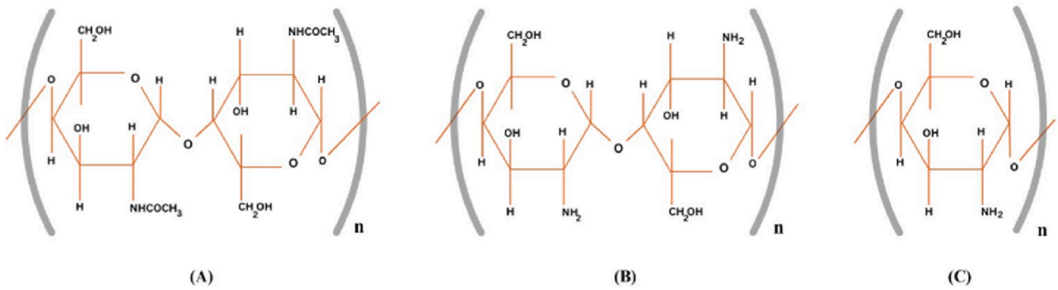

Fig. 4. Chemical structures of A—Chitin, B—Chitosan, and C—Chitooligosaccharide. N refers to the number of the disaccharide units. N corresponding to less than 12 is defined as an oligosaccharide.

in these areas [17].

Waste shells are frequently merely abandoned in landfills or the ocean in poor nations. Disposal may be expensive in affluent nations; for instance, in Australia, it can cost up to USD 150 per tonne [12]. Repurposing chitin and its obtained derivatives for applications in the pisciculture industry is now home to various upcoming methodologies.

Fig. 3 represents the schematic entailing the processing of shell waste from crustaceans to chitosan and its derivatives. There are two methods for the same, namely biological, and chemical. In the biological method particularly—it involves a series of demineralization, deprotonation and decolorization which are catalysed by various proteolytic bacteria [17,18]. As for the chemical method, it is brought about by a series of acid-base treatment steps for the same earlier mentioned three preliminary stages in the process [19]. Given the simplicity of the preparation methods and required conditions, chitosan and its derivatives are inexpensive and easy-to-make compounds [20].

2. Chitin, chitosan, and chito-oligosaccharides

Chitin, first identified as a polymer in 1884 has become one of the most important natural polymers in the world [21]. This polymer has become increasingly popular due to the advantageous properties possessed by its derivatives—chitosan and chito-oligosaccharides. These derivatives have become most popular for their plausible application in the pharmaceutical and the biomedical industry. The properties of the same have been discussed below, with a special focus on their applications in the pisciculture industry.

2.1. Chitin

The most abundant natural biopolymer after cellulose is chitin [21,22]. It is the major structural component of the cuticle in crustaceans, insects, molluscs, fungi, and other organisms [22]. Marine organisms, on the other hand, use it as an essential source of ocean carbon and nitrogen [23]. As depicted in Fig. 4(A), chitin comprises 2-acetamido-2-deoxy beta-D-glucans linked together in a β (1 → 4) arrangement [24]. As a result, it can be characterized as a cellulose derivative containing acetamido residues instead of C2 hydroxyl groups [25].Click or tap here to enter text.Di-saccharides are repeated in the β-glycosidic bonding between GlcNAc residues at the location of the N-acetyl group. It can therefore be regarded as a polymer of two GlcNAcs, or chitobiose [26]. It has been found to have three different forms of crystalline structures called α-chitin (chains run anti-parallel to each other, β-chitin (the chains are packed in a parallel arrangement) and γ-chitin (two parallel chains alternating with a third in an anti-parallel arrangement) [26,27]. The chitin backbone chains are tightly packed together by hydrophobic forces and H-bonds caused by certain symmetries to form crystalline fibrils [28].

Chitin is a nitrogenous, water-insoluble polymer that accumulates in significant quantities in the waste the fishery sector generates due to its slow degradation processes in crustacean cell debris. Several non-specific enzymes, e.g. cellulases, lipases, proteases, and chitosanases, can break this insoluble polymer into water-soluble chitosan and amino glucose [21,28,29].

It can also be hydrolyzed to produce acetic acid and glucosamine, but alkaline solid solutions between 140° and 160 °C cannot dissolve it [29]. The strong positive charge inherent in chitin derivatives has been put to various uses since it bonds quickly, and it is also seen that it is moulded into sheets or films [25]. It is low in immunogenicity, possesses antibacterial and wound-healing characteristics, and is biocompatible, biodegradable, and bioabsorbable [30,31].

2.2. Chitosan

Chitosan is a polysaccharide, β-(1-4)-2-amino-2-deoxy-D-glucopyranose and β-(1-4)-2-acetamide-2-deoxy-D-glucopyranose binding of biopolymer, obtained through the deacetylation of chitin in alkaline media [32]. Fig. 4((B) represents the molecular structure and the aforementioned biopolymer binding. The typical degree of acetylation of chitosan is less than 0.35, which means the chitin deacetylation is 65% [33]. In the form of acetamide in chitin and amine groups in chitosan, chitin and chitosan contain 5%–8% nitrogen. Because of the presence of these groups, chitin and chitosan are favourable for common amine reactions [34].

Generally, the traditional approach and the fermentation process are the two primary ways to generate chitosan. The conventional method uses sources like molluscs, insects, or crustaceans. And the glucan complexes with chitin or chitosan in fungi biomass are utilised for the fermentation method. After the ashes, proteins, colour, and flavour have been removed from the raw materials using various chemical processes, the fermentation process requires a further filtration step. Alkaline deacetylation is the process by which chitin becomes chitosan. Chitosan is purified using multiple techniques, including dissolving, precipitation, centrifugation, and drying [35].

Chitosan is an excellent component for industrial applications because, unlike chitin, it dissolves more readily in aqueous solutions [36].

Chitosan can precipitate negatively charged molecules (like proteins) and particles (like phospholipid complexes) since it is a positively charged polysaccharide [37]. It is thought that this positive charge causes chitosan's antibacterial effect, interacting with the negatively charged membranes of microbes. It can be shown to form gels at low pH levels, which are then utilised as a flocculant, a clarifying agent, a thickening agent, a fibre, a film, a matrix for affinity chromatography columns, a gas-selective membrane, a plant disease resistance agent, an anticancer agent, a wound healing promoter, and an antibacterial agent.

2.3. Chito-oligosaccharides

When the O-glycosidic compounds of chitosan are cleaved by different techniques, chito-oligosaccharides (COS) with different degrees of polymerization and different numbers and arrangement of D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) units are formed [38]. Fig. 4(C) depicts the molecular structure of chito-oligosaccharides. COS often has an Mw of less than 10,000 Da and a DP of 50–55 as it is depolymerized. Chemically, the extent of deacetylation of COS is determined by the molar units of GlcN on its backbone. The degree of deacetylation of the chitosan used as the starting material for its preparation determines the degree of polymerization, which in turn dictates the molecular weight distribution and pattern of N-acetylation [39].

COS is produced using numerous techniques, primarily chemical (using hydrogen peroxide oxidation and acid hydrolysis), physical (via microwave, ultraviolet, and ultrasonic treatment), enzymatic, and electrochemical procedures, as well as composite degrading techniques developed from these techniques [40]. Like the hydrolyzed products of chitosan, chitosan also has better solubility and lower viscosity under physiological conditions due to the shorter chain length and free amino groups in the D-glucosamine units. One

Table 1
This table represents a comparison in fundamental properties of chitin, chitosan, and chito-oligosaccharides.

Sr. No.	Properties	Chitin	Chitosan	Chito-oligosaccharide	References
1.	Source	Derived from marine crustacean shell-waste material, insects, and exoskeleton of invertebrates.	Derived from chitin via enzymatic deacetylation	From chitin and chitosan degradation via chemical or enzymatic methods	[38]
2.	Molecular weight (kDa)	1×10^3-2.5×10^3	100–1200	<5000 Most of them between 200 and 3000	[38,40,43]
3.	Solubility	Insoluble in water, dilute acids, alkalis, ethanol, and other organic solvents.	Insoluble in water	Soluble in water	[44]
4.	Viscosity (cP)	More viscous compared to COS	Intrinsic viscosity is MW dependent—linearly proportional based on the Mark-Houwink equation. For e.g., $\eta = 0.535$ when MW = 20,698	Low viscosity	[39,45,46]
5.	Biodegradable	Yes	Yes	Yes	[36,47]
6.	Toxicity	No	No	No	[40]
7.	Chemical inertness	Inert	Relatively more reactive as compared to chitin	Weak chemical interactions.	[39,48,49]
8.	Anti-microbial property	Yes	Yes	Yes	[31, 50–53]

Table 2
This table summarizes some case study results of chitosan being administered as a dietary supplement for various warm-water fish species.

Sr. No.	Chitosan Dosage	Feeding Period	Fish Species	Result	References
1.	2–10%	28 days	Hybrid tilapia *(Oreochromis niloticus × Oreochromis aureus)*	Decreased weight gain and increased the feed conversion ratio (FCR)	[61]
2.	4 g/kg	56 days	*Oreochromis niloticus*	Promoted weight gain (BWG) rate and specific growth rate (SGR)	[62]
3.	5 g/kg	60 days	Tilapia	Increased rate of body weight gain (BWG), specific growth (SGR) and feed conversion ratio (FCR).	[63]
4.	10–20 g/kg	75 days	Gibel carp *(Carassius gibelio)*	Reduced growth performance	[64]
5.	0–0.2 g/kg	75 days	Sea bass (*Dicentrarchus labrax*)	Increased of the average daily weight gain and specific growth rate	[65]
6.	1–5 g/kg	70 days	Loach fish (*Misgurnus anguillicadatus*)	Significantly increased body weight gain, specific growth rate, and condition factor	[66]
7.	0–2 g/kg	60 days	Caspian kutum (*Rutilus kutum*)	No effect of final weight, SGR, and condition factor	[67]
8.	5–20 g/kg	60 days	Asian seabass (*Lates calcarifer*)	Increased red blood cells (RBC), white blood cells (WBC), total serum protein, albumin, and globulin	[68]
9.	1, 5 and 10 g/kg	12 weeks	Loach fish (*Misgurnus anguillicadatus*)	Increased activity of phenoloxidase, superoxide dismutase (SOD) and glutathione peroxidase (GPx)	[66]
10.	0, 2, 4, 6 and 8 g/kg	56 days	Tilapia	Induced the activity of SOD, catalase (CAT) and the mRNA levels of SOD, CAT, GPx and nuclear factor erythroid 2-related factor 2.	[63]

amino group and two hydroxyl groups are present in the repeating glycoside residues of COS, which makes them weakly cationic polymers [41]. Life-threatening diseases such as cancer, heart disease, diabetes mellitus, and severe infections can be treated and prevented with the help of COSs because of their potential biological activity against bacteria, fungi, tumours, immunity, antioxidants, and inflammation [42].

Table 1 represents a summary of the elementary properties of the three molecules discussed above. A comparison has been drawn between their source, molecular weight, solubility, viscosity, biodegradability, toxicity, chemical inertness, and antimicrobial properties. It is vital to grasp these differences as they play a major role in their respective applications.

3. Applications in the pisciculture industry

Farming of numerous warmwater fish species, for example tilapia, is faced by the challenge of bacterial pathogenic invasion which lead to high mortality rates in fish and heavy economic loss [54]. Interestingly, important freshwater ornamental fish species such as goldfish and zebrafish also face the same challenge [55]. Administration of high doses of antibiotics to the cultured fish leads to accumulation of toxic residues in the tissues. Cooking the fish for consumption by humans does not affect these residues, leading to bioaccumulation [56,57].

Recent studies have shown the sustainable nature of chitosan and its derivatives. It has been reported to impart immunostimulatory effects and improvement in fish growth, depending on the species and growth stage. Such properties help marine life in increasing their average survival rates, with regards to such sustainable alternatives. Below summarized are various formulations of administration and the respective effects that were observed.

Table 3
This table summarizes some case study results of chitosan nanoparticles being administered as a dietary supplement forTilapia fish.

Sr. No.	CSNP Dosage	Feeding Period	Fish Species	Result	References
1.	5 g/kg dry diet	60 days	Tilapia	Increase in body weight	[68]
2.	0–2 g/kg	45 days		Significantly increased body weight gain, specific growth rate, and condition factor	[69,70, 76]
3.	1–5 g/kg	70 days		Significantly increased body weight gain, specific growth rate, and condition factor	[65]

Table 4
This table summarizes some case study results of chitin and chito-oligosaccharides being administered as a dietary supplement for various warm-water fish species.

Sr. No.	Fish Species	Effect caused by inclusion of Chito-oligosaccharides	References
1.	Juvenile largemouth bass (*Micropterus salmoides*) Striped catfish (*Pangasianodon hypophthalmus*) Nile tilapia (*Oreochromis niloticus*) Tiger puffer (*Takifugu rubripes*) Koi (*Cyprinus carpio koi*) Silverfish (*Trachinotus ovatus*)	Enhanced body weight gain rate, hepatosomatic and intestosomatic index, specific growth rate and feed conversion ratio	[71–75,77]
2.	Hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) Rainbow trout (*Oncorhynchus mykiss*)	No significant effects on weight gain, feed conversion and the survival rate	[78,79]
3.	Juvenile largemouth bass (*Micropterus salmoides*), Nile tilapia (*Oreochromis niloticus*), Striped catfish (*Pangasianodon hypophthalmus*) Mrigal carp (*Cirrhina mrigala*) Seabass (Lates calcarifer)	Significant stimulation of respiratory burst activity, phagocytic activity, and lysozyme activity. Fish fed on the control diet perished at a rate of 70%, but fish given chitin derivative supplements perished at a rate of 20% after infection.	[72,74,75,77, 78–83]

3.1. Food supplementation

Several studies have been carried out for evaluation of chitosan and its derivatives for their sustainable use in aquaculture/pisciculture for their immunostimulatory effects, antibacterial activity and non-toxicity [58,59]. However, there has been more focus on the applications of chitosan and its oligos as compared to chitin since dissolution of chitin is a concern as it dissolves only in certain solvents that are highly toxic. Chitosan being easily dissolvable in solvents like acetic acid, and its free amino groups make it an ideal molecule for advantageous effects [60]. A similar argument applies for chitosan oligosaccharides as well. Thereby, with regards to the numerous properties listed in the above sections, the following sections contain a summary of studies carried out in terms of these derivatives and their observed effects on various fish species.

3.1.1. Dietary supplementation with chitosan

Existing literature suggests multiple observed effects of dietary supplementation with chitosan like reduced weight gain, improved feed conversion ratio, and improved growth performance. Table 2 is a summary of the effect of different chitosan dosage and the observed result for the same, with varying feeding period depending on the fish species.

Chitosan's antioxidant action is facilitated by its ability to bind metal ions or scavenge free radicals by donating hydrogen or one pair of electrons [69]. In a study, when Tilapias were subjected to a diet of 4 g/kg chitosan, various effects were observed regarding its antioxidant property. Some of them were—increased superoxide dismutase and other enzymes like lysozyme and catalase activity. It not only improved disease resistance capability against *Aeromonas hydrophila*, but it also decreased lipid, cholesterol and tri-acylglycerol levels [58]. Similarly, Chen et al., 2014 reported that oral administration of chitosan was recommended for better survival of gibel carp. With a dietary supplementation of 4 g/kg of chitosan, an enhancement in the phagocytic activity of the blood leukocytes and the complement haemolytic activity was observed. A 7.5–10 g/kg dosage of chitosan in the diet enhanced protection against *A. hydrophila* pathogenicity [60].

Yan et al., 2017 reported another study which exploited the properties of chitosan as a dietary supplement for *Misgurnus anguillicaudatus*. Increasing administered concentration led to reduced gut lipid content and reduced mRNA expression levels of intestinal lipase and FA (fatty acid) binding protein-2. Chitosan-supplemented fish were observed to have a higher mucus lysozyme activity, while the expression of other enzymes like superoxide mutase, glutathione peroxidases and catalases also showed an increase. Overall, chitosan supplementation improved growth performance, antioxidant status, and immunological response in loaches [67].

3.1.2. Dietary supplementation with chitosan nanoparticles

Tilapia being one of the most important warmwater fish species with high economic value, there has been a wide range of research conducted on strengthening its mucosal immunity and making it more resistant. Table 3 lists the *in-vitro* effect of varying dosage of chitosan nanoformulation supplementation on tilapia for different feeding periods.

Other studies demonstrated that chitosan nanoparticles also improved innate immunity by increasing fish haematological parameters like lysozyme malondialdehyde, and respiratory burst activity [69,71]. For instance, research of the effects of chitosan nanoparticles combined with thymol on tilapia growth, liver, and kidney function, was conducted where it showed that there was an increase in feed efficiency and protein efficiency ratio [72]. The effects on final weight, weight gain, and specific growth rate were moderate at the same time by the implementation.

Tawwab et al., 2019 hypothesized that the increased height of the small intestine's intestinal villi, which in turn enhances feed intake and nutrient absorption, may be responsible for the improvement in fish growth. Inhibiting possible infections, increasing the number of helpful bacteria, and enhancing microbial enzyme activity in the fish gut may all work together to promote feed digestibility and nutrient absorption/assimilation by the inclusion of chitin and chitosan [69]. Naiel et al., 2020 reported that Vitamin C and ChNP together in the diet demonstrated immunomodulatory effects against Nile tilapia toxicity from Imidacloprid. Supplementing with ChNP and vitamin C enhanced growth and feed consumption in pesticide-polluted water. Antioxidant status and non-specific immunity both improved in ChNP and vitamin C groups subjected to imidacloprid toxicity [72].

3.1.3. Dietary supplementation with chitin and chito-oligosaccharides

Chitin and chito-oligosaccharides have a multitude of effects in various fish species which have been summarized via Table 4.

According to other study by Liu et al., 2014, chitin and chito-oligosaccharides also stimulate the generation of nitric oxide, the gene expression of induced nitric oxide synthase (iNOS), leukocyte count, and complement activity [81].

Based on a study by Tawwab et al., 2019, it was shown that Nile tilapia responded better to chitosan nanoparticles as a dietary supplement in terms of final weight increase and feed conversion ratio than to the diet containing chitosan alone. This comparison shows that nanoscale chitosan performs better than its regular form. These results could be attributed to the fact that the chitosan nanoparticles were present in the bloodstream for a long time, which helped to increase its high bioavailability, as well as the fact that CSNPs have better nutrient assimilation and absorption in fish than regular chitosan has a dietary supplement [].

3.2. Drug delivery and vaccination

In recent years, chitosan has become increasingly popular and most researched for its gene and drug delivery applications in fish biotechnology. Chitosan, being a nanoscale compound with straightforward and gentle preparation conditions, is extremely efficient for drug loading. Hence, chitosan has been used for loading a variety of bioactive compounds for its applications in the fish farming industry. Additionally, it has been observed that loading of compounds into chitosan boosts the biological effects of the same [84].

Depending on the loading material, the properties of chitosan as a carrier are prone to change. Subsequently extensive research has been carried out to determine these characteristics that affect the drug release and the encapsulation efficiency of the same.

3.2.1. Gene delivery

The multiple primary amine groups present in chitosan are protonated at an acidic pH allowing interaction with the negatively charged nucleic acids. With regards to this property, CS-DNA complexes have great therapeutic potential as they touch most of the necessary characteristics. Thereby, chitosan is one of the most effective non-viral gene delivery systems [85].

Existing literature indicates that the diameter of the CS-nanoparticles increases on encapsulation with plasmid DNA, rather than with just DNA. Usually, on loading plasmid DNA, the zeta potential decreases. However, an exceptional case was reported by Rather et al., 2016, which showed an increased zeta potential value of the kiss-peptin-10 loaded CS-nanoparticles. The Catla fish injected with this formulation showed a notable rise in the gene expression. It showed a DNA encapsulation efficiency of over 80%, indicating that chitosan can load a higher mass of DNA, which may have numerous benefits for the aquaculture and pisciculture industry [86].

Kumar et al., 2008, formulated a vector containing the porin gene of the fish pathogen *Vibrio anguillarum*, known to induce significant mortality. This formulation was supervised via the oral route by using CSNP (chitosan nanoparticle) encapsulation—giving a DNA vaccine. The expression of this gene was observed, both *in vivo* and *in vitro*, in the kidney cell line of sea bass and in the fish. A survival rate of up to 46% was observed [87].

Furthermore, OmpA has been reported as a potential vaccine candidate to be prevalent in *P. salmonis*, *M. viscosa*, *A. salmonicida* (as porin OmpAI and OmpAII), and *V. anguillarum* (as porin OmpA). In a study by Dubey et al., 2016, recombinant OmpA encapsulated in chitosan nanoparticles was administered orally to *Labeo fimbriatus* and was demonstrated to provide 73.3% survival when infected against *E. tarda* [88]. The infections caused by *V. alginolyticus* and *E. tarda* were prevented with the help of a novel hybrid OmpA that was created via DNA shuffling. In another study carried out by Maiti et al., 2011, it was reported that common carp vaccinated with the recombinant OmpA protein also showed 54.3% of relative percentage survival against *E. tarda*, thereby resulting in a highly significant amount of antibody production in immunized fish [89]. Thus, the OmpA protein has been examined and identified to be highly immunogenic in fish.

In another study by Du et al., 2010, the factors that affect the stability of the CSO-SA (Stearic acid grafted Chitosan oligosaccharide) cationic polymer were characterized. The CSO-SA was tested for delivering the fish sperm DNA at different molecular weights of the oligosaccharide to find the best fit formulation. The results showed that at higher molecular weights of CSO—with higher degree of substitution of the amino groups, the acidity levels of CSO-SA decreased whereas at lower molecular weights, the acidity was less affected. This indicated that the complex was the most stable in a lower pH environment (Du et al., 2010).

In a study by Leung et al., 2022, polyplexes (LMWCSrNP) with gamma-polyglutamic acid were created with a new low-molecular-weight chitosan (LMWCS). Delivery of LMWCSrNP(IRF9S2C) into zebrafish liver cells and larvae stimulated the expression of interferon-stimulated genes, suggesting an improvement of the innate immune system, and consequently representing a prospective nucleic acid delivery mechanism [90].

3.2.2. Recombinant protein delivery

Fish vaccination for infectious diseases is a major issue in the pisciculture industry. This can be improved by using antigenic proteins derived from various bacteria and viruses. Dubey et al. (2016) reported the use of a recombinant OmpA obtained from *Edwardsiella tarda* which was encapsulated within chitosan nanoparticles (referred to as NP-rOmpA) and administered for oral vaccination of a cyprinid fish (*Labeo fimbriatus*). The results showed an increase in the antibody production level in the fish and elevated protection against the pathogen *Edwardsiella tarda*. A decrease in mortality rate of the fish was also observed [88]. Gao et al. (2016) reported the use of chitosan nanoparticles to encapsulate the ECPs of *Vibrio anguillarum*. This construct was used as an oral administration for immunization in flatfish (Turbot). The results showed an increased level of specific antibodies and an inflated concentration of lysozyme activity. The results also showed enhanced adaptivity and non-specific immune response as well [91]. Recently, Huang et al., 2021 explored a potential approach for bulk oral vaccination of varps against Koi herpesvirus (KHV) ionfection is through an oral probiotic vaccine expressing the Koi herpesvirus (KHV) ORF81 protein delivered via Chitosan-Alginate capsules [92].

3.2.3. Recombinant bacteria encapsulation

A vital challenge in the aquaculture industry is the vaccination of marine life against distinguished pathogens. However, chitosan and its derivatives have shown promising results as a potential drug carrier by enhancing delivery and vaccine efficacy for fish.

A study by *Zhang* et al. *(2019)* was carried out in which chitosan was tested with the inactivated form of *Edwardsiella ictaluri* and ISKNV (acronym of Infectious Spleen and Kidney Necrosis Virus) in *Pelteobagrus fulvidraco* (yellow Catfish) and *Siniperca chuasi* (Chinese perch), respectively. The results indicated a boost in liver and spleen tissue protection, higher survival rates of the fish and improved immune response [93].

Another piece of literature by Halimi et al. (2019) reported increased survival rates of *Oncorhynchus mykiss* (Rainbow trout) and expression of immune-related genes. The species was immunized against the infection caused by *Streptococcus iniae* and *Lactococcus garvieae*, via vaccination containing coated formulation of chitosan and alginate [94]. Recently, Chang et al., 2023 reported that in grass carp, oral administration of recombinant *Lactobacillus casei* (rLc) expressing VP56310-500 and adjuvant flagellin C via alginate-chitosan (SA/CS) microcapsules significantly improved immunological defence against the grass carp reovirus (GCRV) infection. Grass carp that received the oral vaccination with SCL-56310-500C showed a significantly greater survival rate against GCRV infection (58% against 24% in the control) [95].

Similarly, a mixture of chito-oligosaccharides and inactivated form of the pathogen *Vibrio anguillarum,* yielded a vaccine that notably decreased the mortality rate in zebrafish due to *V. anguillarum* [96]. While a combination of chito-oligosaccharides and inactivated form of *Vibrio harveyii*, did not just report a remarkable increase in survival rates but also showed a surge in the expression of IL-16 and other major immune-related genes [97].

3.2.4. Micronutrients

It is important to establish proper nutritional sources for all marine life. Oftentimes one of the challenges faced by the pisciculture industry is the uptake of micronutrients by marine life, especially fish. In absence of the right supplementation, the fish show noticeable symptoms of deficiency, which could also lead to eventual death [98]. Regarding the drug delivery properties of chitosan, researchers are trying to explore the same as a possible solution for nutrient uptake. Chitosan as a carrier of essential micronutrients for marine life can have multiple advantages with connection to its antimicrobial properties.

On supplementation of CS-Ag nanocomposites exhibited alteration in the microbiota of zebrafish and its gut morphometric dimensions. The same formulation showed upregulation in the immune-related gene expression of *Bacteroidetes* and *Fusobacteria* taxa [99].

Similarly, the CS-Ag nanocomposites increased survival rates of *Dicentrarchus labrax* (European sea bass larvae) that was infected with *V.anguillarum* [100]. For artificial fish breeding in fish farms, delivery of vital compounds is crucial. Given the non-toxic nature of chitosan, it allows sustainable release of these biomolecules and its cellular uptake [101]. Vitamin C encapsulated within chitosan nanoparticles has been proven by a study to show a sustained release in the intestine, stomach, etc in *Oncorhynchus mykiss* (Rainbow trout) post oral administration of the formulation [102].

Similarly, another study by Fernandez et al., 2014 witnessed a higher antioxidant activity with zero toxicity in culture medium containing ZFL cells. The nanocomposite also showed penetrative ability in the intestinal epithelial layer of *Solea senegalensis* [103]. Thereby, these studies have widened the scope for studying optimal nutrition provision strategies in aquaculture by using nanotechnology for oral drug administration.

3.3. Antioxidant and antimicrobials

Since Alan and Hardwiger [104] initially claimed that chitosan had extensive antibacterial properties, researchers have been trying to explore the antimicrobial property of this substance as well as its commercial viability [50]. Literature suggests various potential mechanisms via which chitosan exhibits its antimicrobial property. Some of the proposed mechanisms are—with regards to its positive charge it could disrupt the cell membrane and increase the permeability of the membrane, inhibition of mRNA thereby affecting

Table 5
This table summarizes various case studies highlighting the efficacy of chitosan as a seafood preservative, subjected to the samples in different forms.

Sr. No.	Product	Method of study	Chitosan quality	Properties studied	Result	References
Coatings						
1.	Snakehead fish; 2 ± 0.5 °C, 5 months	Chlorogenic acid and chitosan solution (2%, w/w) were combined to create the coating solutions.	Deacetylation degree ≥90%	Antioxidant, antimicrobial, and sensory properties	Fish fillets coated with chitosan had antioxidant and antibacterial capabilities. Adding CGA further increased the antioxidant qualities but had no effect on the hardness of the snakehead fish fillets during preservation.	[112]
3.	Nile tilapia; 4 ± 1 °C for 30 days	Chitosan was dissolved in 1% (w/v) lactic acid, 1% (v/v) chitosan, and 0.1% (v/v) glycerol to create the coating solution.	Degree of deacetylation (60–80%)	Physicochemical and microbiological characterisation	Antimicrobial and antioxidant properties improved the quality of coated fillets. The liquid smoking connected to the chitosan coating induced longer fish shelf life.	[114]
4.	Grass carp fillets; 15 days at 4 °C	5 min of dipping in a solution containing 2% chitosan and 0.1, 0.1, 0.5, and 1% of clove bud essential oil.	Deacetylation degree: 85% Molecular weight: 400 kDa	Sensory	Prevention of a decrease in sensory ratings when storing, decreased production of histidine, volatile oxidation products, off-tasting nucleotides, and trimethyloamine, and 4–8 days longer shelf life.	[115]
Inclusion in Batter						
5.	Fish sticks; Rohu	Addition of 0.5, 1, 1.5 and 2% of chitosan gel during batter mixing	Deacetylation degree: 86% Molecular weight: 76.5 kDa	Antioxidant and sensory	By lowering the oil absorption during frying and by minimising the lipid oxidation during frozen storage of parfried fish sticks, the addition of chitosan gel to batter aids in enhancing the physicochemical quality characteristics of enrobed fish sticks.	[116]
6.	Fish sausages; Nile tilapia	Half of the sausage batter was mixed with a cold, 10% chitosan solution in 1% acetic acid at 4 °C (15 g/kg of the prepared batter).	Deacetylation degree: 91% Molecular weight: 29 kDa	Antimicrobial activity, Sensory	Throughout the storage period, there was a noticeable decrease in the number of yeasts, bacterium, moulds, and coliforms. Furthermore, the odour and taste qualities were noticeably improved.	[117]
Tumbling						
8.	Gutted fresh silver carp carcass, Stored: for 30 days at − 3 °C	Deacetylation degree: 85%	120-min immersion in 2% chitosan solution and 1% acetic acid.	Antioxidant Antimicrobial Sensory	Significantly decreased production of total volatile basic nitrogen and thiobarbituric acid reactive compounds and extension of the shelf-life by 5 days.	[118]

protein synthesis or by acting as a chelating agent for the nutrients [105]. Several such antimicrobial characteristics of chitosan and its nanostructures make them effective antifungal and antibacterial agents against antibiotic-resistant Gram negative and Gram-positive bacteria. Chitosan and its nanocomplexes, such as N-stearoyl O-butylglyceryl/chitosan, have been used for the encapsulation and slow release of fish oil because of their antioxidative and antimicrobial properties. Furthermore, FG/chitosan edible films with the plasticizer hydrophobic D-limonene have improved the films' antibacterial characteristics while providing outstanding mechanical resistance against light and water penetration [106].

In addition to having higher antioxidant properties than unconjugated chitosan, derivatives made by conjugating chitosan with phytochemicals also have good antibacterial effects against several foodborne pathogens and methicillin-resistant *Staphylococcus aureus* (MRSA) [107].

3.4. Fish and seafood preservation

The consumer demand for food items free of artificial food additives is growing, according to recent research on consumer trends [108]. Since the inception of chitosan, scientists have increasingly been proposing novel active packages that are based on the use of active agents with carbon dioxide-emitting/generating, antibacterial, or antioxidant action to increase the shelf life of packaged foods [109]. According to the review by Elsabee et al., 2013, chitosan-based films often feature inherent antioxidant and antibacterial capabilities as well as selective gas permeability, making them suitable substitutes for active packaging matrix [110]. Future developments in food technology will rely on packaging materials that are non-toxic, biodegradable, and biocompatible.

Chitosan has gained enormous popularity across a variety of industries and is being utilised as a healthy, natural food component [111]. In the food industry, chitosan (CS) coating is a nontoxic, attractive, and natural coating material used to reduce lipid oxidation and microbial growth. Furthermore, by releasing antioxidants and antibacterial agents, additives used in edible coating also increase its activity in preservation [112].

Chitosan is a powerful antioxidant and antibacterial agent, and its use in seafood items can alter the shelf life and quality of those goods. The application technique, seafood type, chitosan concentration, and chitosan characteristics including viscosity, particle size, and deacetylation level affect how effective chitosan is [113]. Table 5 highlights multiple studies that have produced distinct results depending on the warm-water fish species tested, the quality of chitosan employed, and the properties assessed. This summary is crucial for drawing a plausible relation between the treatment and its observed effects.

Numerous research studies have demonstrated the usefulness of applying chitosan to various fish products. When chitosan-based preservation techniques are used, sensory quality can be improved while oxidation and microbiological development is slowed down. It should be emphasized that these effects are supported by several authors who have measured oxidation rates and microbial activity with various analytical techniques.

Apart from coatings, inclusion into batter, and vacuum tumbling, chitosan nanoparticles are a viable additive for increasing the effectiveness of chitosan coatings. Chitosan nanoparticle coatings are more successful than coatings with regular chitosan at preventing microbial development, and they enable the preservation of fish fillets and shrimp meat with good sensory values [119]. The storage life of fish fillets wrapped in composite nanofilms could be extended by 6–8 days through sensory evaluation, microbiological analysis, pH, total volatile alkaline nitrogen value, thiobarbituric acid value, colour, texture, and other storage quality indicators, according to a study by Ran et a on the use of chitosan nanoparticles for fish preservation [120]. They can also be used in combination with other additives for synergistic effects toward the preservation of fish [121,122]. In another study, the impact of chitosan gel inclusion in pre-emulsified fish mince (Pangasianodon hypophthalmus) sausages was investigated, and all chitosan gel addition combinations increased the stability of the emulsion—improved colour, texture, and water holding capacity metrics were seen [123].

4. Conclusion and future prospects

Freshwater fish species form the foundation of multipAuthor contribution statement:

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that no potential conflict of interest that could have appeared to influence the work reported in this paper.

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