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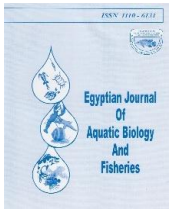

The Effectiveness of Chitosan-Based Fresh Fish Preservation and its Combination with Bioactive Substances: A Review

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ABSTRACT

Fish is a significant source of nutrition for many people globally. Many consumers value this product since it is low-priced and the meat is easily digested. However, the quality of fish degrades easily and rapidly due to enzymatic activity, bacterial activity, and chemical oxidation. Concerns about the health risks associated with using synthetic preservatives have drawn much attention to natural active ingredients that may be able to prolong the shelf life of fresh fish. Chitosan is one of the naturally occurring polymers a preservative to prevent bacterial and oxidation in the fish. This review focused on the effectiveness of chitosan as a preservative for fresh fish and its antioxidant and antibacterial properties. The review also covers the effectiveness of combining chitosan with other bioactive substances in reducing bacterial activity, protein oxidation, and lipid oxidation during fresh fish storage. Chitosan can preserve the freshness of fish during storage. Chitosan has excellent antimicrobial and antioxidant properties. Numerous studies have demonstrated that chitosan's antibacterial and antioxidant activities are directly proportional to its DD and inversely proportional to its MW. However, there is currently insufficient data concerning the relationship between the two features of chitosan and its capacity to preserve fish quality during storage. Chitosan's capacity to keep the freshness of fish can be enhanced by its combination with numerous other natural active compounds. It is intriguing to investigate whether combining chitosan and other natural active compounds is synergistic or additive.

INTRODUCTION

Due to its high nutrient content, including protein, fat, vitamins, and minerals, fish is one of the essential food items used to satisfy the daily nutritional needs of people in many nations throughout the world **(Tilami & Sampels, 2018)**. Additionally, fish meat is easier to digest and relatively low-cost than other types of meat. However, the fish's quality degrades shortly after death, so its shelf life is brief. The decline in fish quality is primarily attributable to extremely rapid microbial activity, naturally or as a result of contamination. Fish is an excellent medium for microbial growth due to its high water activity, close to neutral pH, and high protein content with free amino acids **(Carrión-Granda *et al.*, 2018)**. Also, contributing to the decline in fish quality is

autolysis and enzymatic and non-enzymatic oxidation **(Mei *et al.*, 2019)**. This relatively rapid drop in quality will have negative economic and nutritional consequences. Globally, 10% (10-12 million tonnes) of total capture and aquaculture production is lost owing to fish spoilage **(Socaciu *et al.*, 2018)**. Therefore, precise and swift handling is required to preserve fish quality during storage.

Several techniques are used to preserve fish's quality and freshness during storage, including cooling, non-thermal sterilization, coating, vacuum packaging, edible film, and the addition of preservatives **(Mahmud *et al.*. 2018; Tsironi *et al.*, 2020; Umaraw *et al.*, 2020)**. The cooling technique is considered the most effective and safest for preserving fresh fish. However, cooling alone is insufficient to extend the shelf life of fresh fish. Even with ice box storage, fish quality measures such as TVB-N, TBARS, and total bacteria continued to grow throughout storage **(Abou-Taleb *et al.*, 2018)**, even frozen storage did not prevent this **(Rasul *et al.*, 2022)**. Generally, synthetic preservative treatments have been employed to extend the shelf life of fresh fish. However, people do not like this method of fish preservation, because they worry about the potential health risks of gastrointestinal irritation, allergies, and the cancer-causing properties of synthetic preservatives. This concern promotes the study and implementation of natural preservatives to extend the shelf life of fish **(Mei *et al.*, 2019)**.

Chitosan is one of the natural substances extensively researched for its use as a preservative in various food products, including fish. Chitosan results from the deacetylation of chitin, the second-most abundant natural polymer after cellulose. Most chitin is found in shrimp and crab shells, which have little economic value and are typically discarded in the shrimp and crab processing industries. Multiple studies have demonstrated that chitosan coating on fish meat extends its shelf life **(Chamanara *et al.*, 2013; Rezaabad *et al.*, 2017; Ramírez-Guerra *et al.*, 2018; Rostamzad *et al.*, 2019; Yang *et al.*, 2019; Santos *et al.*, 2020).** Chitosan possesses excellent bioactivity, including antibacterial and antioxidant properties **(Inanli *et al.*, 2020).** Chitosan is found to have antibacterial characteristics **(Goy *et al.*, 2016; Hosseinnejad & Jafari, 2016; Tachaboonyakiat, 2017)** and antioxidants **(Rajalakshmi *et al.*, 2013; Si Trung & Bao, 2015; Avelelas *et al.*, 2019)** sufficient to protect fish meat against microbial activity and enzymatic and non-enzymatic oxidation processes.

Chitosan is not a single polymer; the features of one chitosan are likely to differ from those of another chitosan, if the raw materials or isolation processes differ. The crystalline structure of chitin/chitosan comprises α-, β-, and γ- formations depending on the type of raw material **(Kaya *et al.*, 2016)**, which influence the effectiveness of deacetylation and its antibacterial characteristics **(Jung & Zhao, 2013)**. The primary characteristics of chitosan, the degree of deacetylation (DD), and molecular weight (MW) are strongly influenced by the type of raw material and the method/treatment of isolation. Hence, chitosan has different properties, its uses, like preserving fresh fish, will have different levels of success.

Most studies on chitosan as a fresh fish preservative are conducted by coating and utilizing edible films **(Socaciu *et al.*, 2018)**. Thus, many studies have been done, there is still not enough proof that chitosan is effective at keeping fish fresh, despite the fact, it is being stored associated with the characteristics of chitosan. Several research results cited by **(Inanli *et al.*, 2020)**, indicated that the efficiency of chitosan in safeguarding fish quality depends on DD, MW and concentration of chitosan and the fish species. This paper pursues to present an overview of existing research on the relationship between the primary characteristics of chitosan (DD and MW), packaging and its combination with additives, and its effectiveness as a fresh fish preservative.

REVIEW

1. Resources and preparation of chitosan

Chitosan results from chitin's deacetylation, a natural polymer with the second-highest abundance after cellulose. Chitin and chitosan resources are abundant in crustaceans **(Mesa Ospina *et al.*, 2015)**, fungi **(Ghannam *et al.*, 2016; Santos *et al.*, 2020)** and invertebrates. The features of the chitosan produced are significantly influenced by the type of chitosan resource used **(Kumari *et al.*, 2017)**. Chitin extracted from commercially collected crustaceans, such as shrimp, crabs, and lobsters, is probably the main source for large-scale production of chitin and chitosan. Crab and shrimp meat processing plants generate vast quantities of chitinous materials as waste **(Yadav *et al.*, 2019)**.

Two fundamental processes, deproteination, and demineralization are required to separate chitin from crab shells **(Gadgey & Bahekar, 2017)**. The deproteination process can be accomplished chemically with 4-6% NaOH, enzymatically with protease enzymes, or biologically with protease-producing bacteria such as *Pseudomonas aeruginosa*, *Pediococcus acidilactici*, *Bacillus subtilis*, and *B. firmus* **(Wahyuntari *et al.*, 2011; Pal *et al.*, 2014)**. The demineralization process can be conducted chemically using a strong acid such as HCl 1-2 N or biologically with lactic acid-producing bacteria such as *Lactobacillus plantarum* **(Arbia *et al.*, 2013; Pal *et al.*, 2014)**, *L. salivarius*, *L. paracasei*, and *Serratia marcescens* (**Neves *et al.*, 2017**). Besides, chitin, minerals, and proteins, crustacean shells also contain several pigments. Chlorite, acetone, or peroxide can be used to eliminate the pigments.

The deacetylation process removes the acetyl group from the chitin molecular chain, typically accomplished using a NaOH-based chemical treatment **(Paul *et al.*, 2014)** or by enzymatic *N*-deacetylation utilizing chitin deacetylase **(Kaczmarek *et al*,. 2019)**. Typically, the chemical deacetylation procedure uses conventional heating, but microwave and autoclave heating methods are more effective **(Ibrahim *et al.*, 2019)**. Although, crab shells and shrimp shells have become crucial raw materials for the commercial production of chitin and chitosan on a global scale, according to **Abo Elsoud & El Kady (2019)**, fungal mycelia are an excellent source of chitosan than crustaceans. Consequently, in recent years, the trend of studies exploring the possibilities of using fungi and other non-crustaceans as raw materials for chitosan manufacturing has increased **(Philibert *et al.*, 2017)**.

Depolymerization is often used to make chitosan with smaller particles, along with deproteination, demineralization, depigmentation, and deacetylation. Depolymerization can be done chemically with HCl **(Qandil *et al.*, 2018; Affes *et al.*, 2021)**, H_2O_2 **(Tanasale *et al.*, 2019)** and CH_3COOH **(Santoso *et al.*, 2020)**. While, enzymatically with cellulase **(Jung and Zhao 2013; Rokhati et al. 2018),** xylanase, and glucanase **(Águila-Almanza et al. 2019),** or physically with microwave irradiation **(Wasikiewicz and Yeates 2013; Jo et al. 2019)**.

2. Important physicochemical characteristics of chitosan

The most important physicochemical characteristics of chitin/chitosan in its application are the degree of deacetylation (DD) and molecular weight (MW). These two physicochemical features significantly impact chitosan's bioactivity, particularly its antibacterial capabilities **(Mellegård *et al.*, 2011; Goy *et al.*, 2016; Hosseinnejad & Jafari, 2016; Tachaboonyakiat, 2017).** The degree of deacetylation indicates the number of free amine groups ($-NH_2$) in polysaccharides and is used to differentiate chitin from chitosan. Deacetylation of chitin into chitosan intends to transform the *N*-

acetyl group (-N-$COCH_3$) into an active amine group (–NH_2), which is believed to have a significant role in numerous usage of this natural substance. The degree of deacetylation defines the ratio of the number of amine groups to the *N*-acetylamide groups in chitosan, which is affected by the method and conditions of the deacetylation process **(Moura *et al.*, 2015; Nouri *et al.*, 2016).**

3. Bioactivity of chitosan

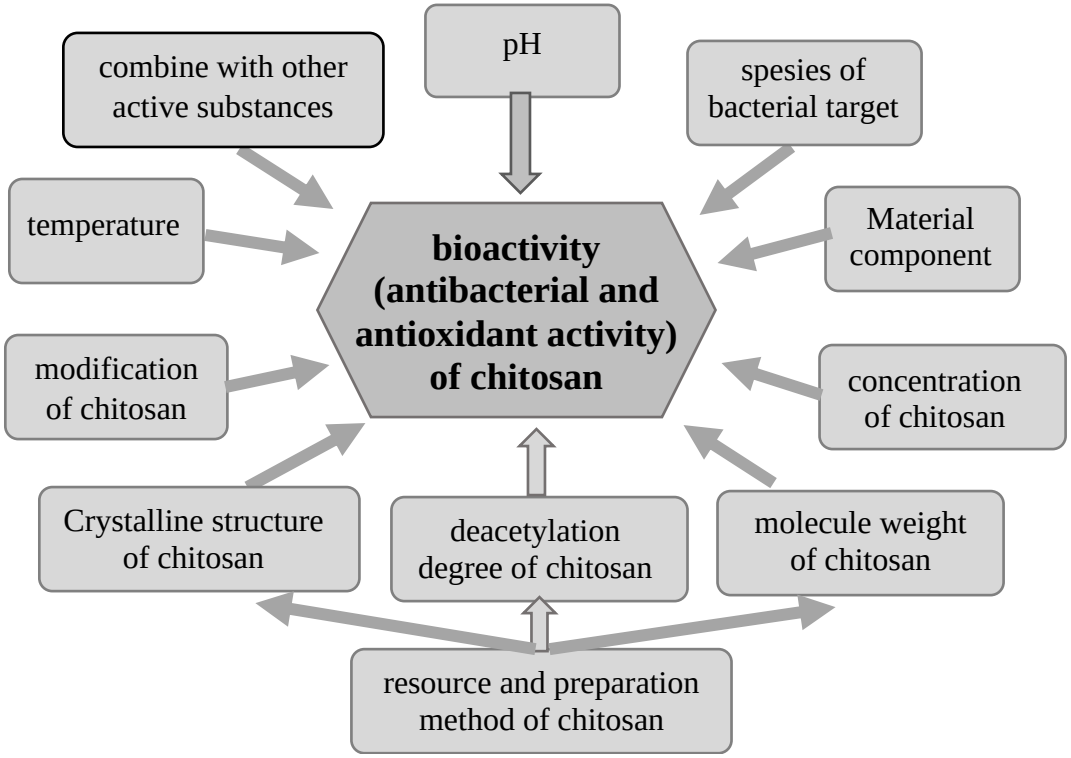

Fig. 1. Different factors which affect the bioactivity of chitosan

Chitosan's bioactivity is directly tied to the active groups it contains. Chitosan has a -NH_2 (amine) functional group attached to the C2 position and a -OH- (hydroxyl) functional group attached to the C3 and C6 positions **(Inanli *et al.*, 2020)**. It is essential to investigate chitosan's antibacterial and antioxidant characteristics for its usage as a fresh fish preserverer. Chitosan's bioactivity is affected by various circumstances, as demonstrated in Fig (1). Chitosan's bioactivity can be changed by its properties, especially its MW and DD, its crystalline structure, its concentration, the microorganisms it is meant to affect, and the temperature and pH of the medium.

3.1. *Antibacterial properties of chitosan*

Chitosan has good antibacterial properties and can be used to protect food products **(Van Toan *et al.*, 2013; Malinowska-Panczyk *et al.*, 2015; Erdem *et al.*, n.d.; Cauhan *et al.*, 2017; El-Dahma *et al.*, 2017)**. The mechanism by which chitosan inhibits bacterial growth has been intensively explored **(Siddique *et al.*, 2020).**

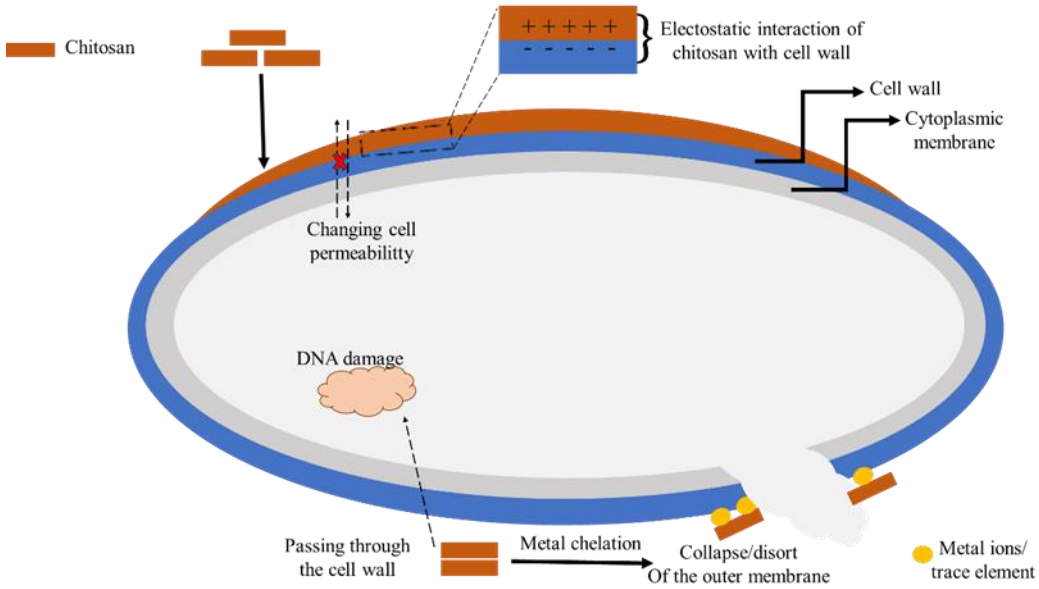

Fig. 2. Schematic representation of antibacterial mechanisms of chitosan (**Hossiennejab & Jafari, 2016**)

One of the theories that most people agree with is the interaction between the cationic group of chitosan ($-NH_3^+$) and the anionic groups on the surface of bacterial cells Fig. (2). Low concentrations of chitosan solutions can absorb water or gas from bacterial cell membranes that cause intercellular leakage **(Kulawik *et al.*, 2020; Junianto *et al.*, 2021)**. Chitosan solutions with a high concentration will create a build-up of chitosan on cell membranes and disrupt microbial metabolism **(Jeon *et al.*, 2014; Tachaboonyakiat, 2017).** The greater the chitosan concentration, the greater the inhibitory power of the chitosan solution **(Cauhan *et al.*, 2017).** Another hypothesized method involves the positive charge of chitosan ($-NH_3^+$) interacting with bacterial DNA cells to limit the synthesis of mRNA and protein **(Tachaboonyakiat, 2017; Yilmaz Atay, 2019; Kulawik *et al.*, 2020)**. This process demonstrated that chitosan's antibacterial properties are tightly tied to its **DD (Barleany *et al.*, 2020). Tsai *et al.* (2002) and Malinowska-Panczyk *et al.* (2015)** demonstrated that the higher chitosan's DD, the higher its antibacterial ability. Variances also influence chitosan's antibacterial activity in resources (raw materials) and isolation techniques **Tsai *et al.* (2002).** Moreover, bacterial species influence the antibacterial action of chitosan. According to **Vieira *et al.* (2019)**, gram-positive bacteria are typically more susceptible than gram-negative bacteria, likely because the cell walls of gram-positive bacteria are much simpler than those of gram-negative bacteria.

The research conducted by **Tamara *et al.* (2018)** on the correlation between the molecular weight (MW) of chitosan and its antibacterial activity against *E. coli* and *B. cereus* revealed that, there was almost no variation in the minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC) values of chitosan with varying MW (80, 200, 500, and 1500 kDa). **Kaya *et al.* (2016)** examined the antibacterial activity of low molecule weight (LMW) and medium molecule weight (MMW) chitosan and determined that there was no significant difference between the two forms of chitosan in inhibiting the growth of multiple bacterial species. According to the findings, there is no correlation between the antibacterial properties of chitosan and its molecular weight (MW). A relatively different picture is presented by **Badawy *et al.* (2016)**, who used chitosan with varying molecular weights (22, 32, 64, 127, 203,

…, 846 kDa), finding that the antibacterial activity of chitosan tended to decline slightly with increasing molecular weight.

By modifying functional groups, such as phenolic groups, chitosan's antibacterial activity can be increased **(Hassan *et al.*, 2018)**. Chitosan's size can also be altered to create nanoparticles (**Alqahtani *et al.*, 2020; Chandrasekaran *et al.*, 2020)**. According to most research findings, chitosan with a nano-size offers superior antibacterial characteristics than chitosan with a high MW. The antibacterial activity of chitosan depends on the tested bacterial species. Gram-positive bacteria are generally more susceptible to chitosan's bactericidal effects than Gram-negative bacteria. Research by **Abdeltwab *et al.* (2019)** compared the minimum inhibition concentration (MIC) and minimum lethal concentration (MLC) values of chitosan nanoparticles with regular chitosan (LMW and HMW) against several types of bacteria. Their findings demonstrated that chitosan nanoparticles had much lower MIC and MLC values than regular chitosan. The research findings obtained by **Divya *et al.* (2017)** were comparable. However, **Tamara *et al.* (2018**) found no difference between the MIC and MBC values of chitosan nanoparticles and regular chitosan against E. *coli* and *B. cereus* bacteria.

It has been demonstrated in several studies that adding chitosan to other naturally occurring antibacterial substances increased their antibacterial effectiveness **(Lee, Dae-Sung *et al.*, 2013). R**esearch conducted by **Raphaël & Meimandipour (2017)** showed that the MIC and MBC values of chitosan and essential oil combined were significantly lower against a variety of bacteria and fungi than those of chitosan and essential oil evaluated separately. According to a research by (**Malinowska-Panczyk *et al.*, 2015)**, chitosan and gelatine combined were more effective at inhibiting bacteria than chitosan alone. **Saloko *et al.* (2014)** found that combining chitosan with liquid smoke showed a broader inhibittory zone than chitosan alone. In studies by **Badawy *et al.* (2016),** chitosan's MIC value against various bacteria was significantly decreased when combined with monoterpenes (geraniol and thymol). Chitosan's ability to reduce the number of bacteria is significantly enhanced when combined with polylactic acid **(Chang *et al.*, 2021**).

3.2. Antioxidant activity of chitosan

Chitosan, in conjunction with having antibacterial properties, also has antioxidant properties **(Kurniasih *et al.*, 2018; Zaghbib *et al.*, 2022; Kusnadi *et al.*, 2022)**. However, it does not have the same level of antioxidant activity as natural antioxidants like ascorbic acid or synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), or propyl gallate (PG) **(Rajalakshmi *et al.*, 2013; Si Trung & Bao, 2015; Avelelas *et al.*, 2019)**. Chitosan can be an antioxidant to preserve foods from oxidative processes **(Hromis *et al.*, 2017)**. The antioxidant activity of chitosan was reported to be correlated with its MW, DD, and raw materials **(Younes & Rinaudo, 2015).** Chitosan with low MW has more potent antioxidant activity than chitosan with high MW (**Sugiyanti *et al.*, 2018)**. The higher the WM, the stronger the intramolecular bonds, thereby reducing the antioxidant activity of chitosan ***(Hromis et al.*, 2017)**. The antioxidant activity of chitosan can be enhanced by the formation of its salts **(Charernsriwilaiwat *et al.*, 2012),** modification **(Abd El-Rehim *et al.*, 2012; Wan *et al.*, 2013; Tamer *et al.*, 2016; Li *et al.*, 2019a).** In addition, to the combination with other natural ingredients such as glucose **(Mahae, et al. 2011)**, liquid smoke and maltodextrin (**Saloko *et al.*, 2014**), *Eucalyptus globulus* essential oil **(Hafsa *et al.*, 2016)**, starch and polyphenols **(Talón *et al.*, 2017).**

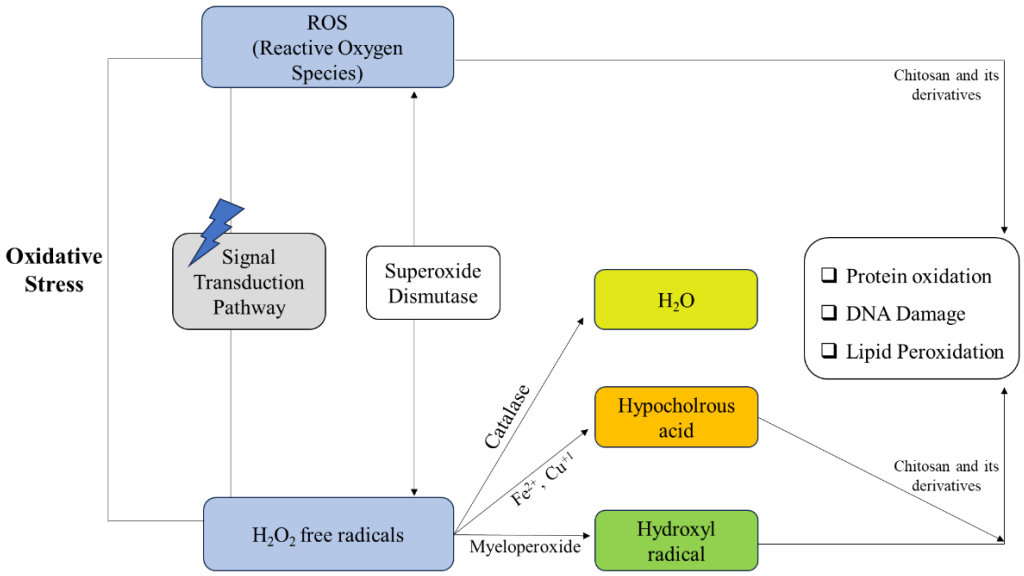

Fig. 3. Antioxidant mechanism of chitosan (**Riaz Rajoka *et al.*, 2019**)

Schematic representation of chitosan antioxidant mechanism to scavenge free radical showcased in Fig. (3). Reactive oxygen species (ROS), including superoxide radicals ($O_2•^-$), hydroxyl radicals (•OH), and hydrogen peroxide (H_2O_2), lead to oxidative stress. ROS damages most biomolecules such as lipids, proteins, amines, lipoproteins, carbohydrates, and DNA at high concentrations. As a natural antioxidant, chitosan has demonstrated significant ROS antioxidant capacity **(Avelelas *et al.*, 2019; Riaz Rajoka *et al.*, 2019)**. The antioxidant activity of chitosan may occur because its free amino groups react with free radicals, resulting in stable macromolecular radicals and ammonium groups **(Riaz Rajoka *et al.*, 2019; Inanli *et al.*, 2020)**. Thus, chitosan's DD directly correlates with its antioxidant activity. Although chitosan has less antioxidant activity than ascorbic acid, it significantly boosts the activity of antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase and lowers malondialdehyde when added **(Charernsriwilaiwat *et al.*, 2012; Riaz Rajoka *et al.*, 2019).**

4. Changes in fish quality during storage

Pre-rigor, rigor-mortis, and post-rigor are the stages typically used to categorize changes in fish quality following death. Enzymatic oxidation processes in the fish's body break-down fats, proteins, and carbohydrates, while it is still alive to produce carbon dioxide and energy. The enzymatic reaction keeps going after the fish dies, but because the oxygen supply is cut-off, the reaction becomes anaerobic. In the early stages of fish death, carbohydrates (glycogen) will break-down into lactic acid, which accumulates in the fish meat so that it can cause protein denaturation. Fish start to produce transparent mucus that covers their entire body (*Hyperemia*), which is the perfect environment for spoilage bacteria to proliferate. The species of fish, the type of muscle fiber, and the storage circumstances all affect the characteristics of fish rigor mortis **(Wang *et al.*, 2019; Tavares *et al.*, 2021).**

4.1 *Proteolysis*

Proteins are divided into smaller polypeptides or amino acids by a process known as proteolysis. Enzymatic processes, both in the fish's body and from spoilage bacteria, cause the proteolysis of dead fish protein. Ammonia, trimethylamine (TMA),

and formaldehyde are produced during this proteolysis process, giving fish meat a foul odor and taste **(Tavares *et al.*, 2021)**. The rate of fish deterioration can be measured as total volatile base nitrogen (TVB-N). The maximum acceptable TVBN value is 25mg/100 grams **(Siddique *et al.*, 2020)**. Numerous studies have demonstrated that when a fish's bacterial population grows, so does the TVB-N value. Chitosan and bioactive substances can slow the increase in TVB-N value rate while inhibiting bacterial growth **(Ahmed *et al.*, 2017; Ramírez-Guerra *et al.*, 2018; Rostamzad *et al.*, 2019; Yang et al. 2019; Meherpour *et al.*, 2020).**

4.2. *Lipid oxidation/lipolysis*

In conjunction with having a high amount of necessary amino acids in its protein, fish also has a high amount of polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and alpha-linolenic acid (ALA), particularly in marine fish. These substances have significant anti-inflammatory effects, guard against cancer and heart disease, and promote brain development ***(Mei et al.*, 2019)**. Contrarily, PUFA compounds are vulnerable to oxidation processes that may result in unfavorable changes to flavor, odor, color, and texture **(Siddique *et al.*, 2020)**. Despite the fact, the fish are still alive, excessive oxidative reactions are prevented by antioxidants synthesized in their metabolism. However, after the fish die, there is no longer any defence against oxidative damage. The initial reaction of lipid/fatty acid oxidation produces hydrogen peroxide, which has no impact on the taste of the fish. However, other oxidation processes will result in aldehydes and ketones, which create rancidity and a fishy smell **(Tavares *et al.*, 2021).** Enzymatic free fatty acid lipolysis increases lipid oxidation products as well. A test utilizing thiobarbituric acid (TBARS) can determine the formation of these secondary oxidizing compounds **(Dehghani *et al.*, 2018; Tavares *et al.*, 2021).** For an excellent sample, the TBA value should not exceed 2-3mg MDA/Kg; for a good sample, it should not exceed 5mg MDA/Kg **(Vieira *et al.*, 2019).** The maximum allowable intake is about 7-8mg MDA/Kg **(Siddique *et al.*, 2020).** The rate of lipid oxidation can be reduced by using various bioactive substances that contain antioxidant compounds such as polyphenols, ascorbic acid, essential oils, and polysaccharides **(Mei *et al.*, 2019; Inanli *et al.*, 2020).**

4.3. *Microbial decay*

During storage, microbial activity is the most crucial factor affecting the quality of fresh fish. Fish muscles are free of microorganisms while alive, but after they die, the microbes in the fish's skin, stomach, and gills contaminate the muscles. The conditions inside a fish's body are suitable for microbial growth, so they grow and change quickly. However, not all bacteria play a part in fish spoilage; only a small number of microbes, known as specific spoilage organisms, are responsible for spoilage **(Mei *et al.*, 2019).** The spoilage bacteria *Pseudomonas* and *Shewanella* are commonly found in various fish **(Gram & Huss, 1996; Zhang *et al.*, 2021).** Spoilage bacteria degrade proteins/amino acids and generate substances that emit a foul odor and, at specific concentrations, are toxic **(Tavares *et al.*, 2021).**

5. Application of chitosan as a preservative for the quality of fresh fish

Fresh fish products are among the commodities that require appropriate handling methods and technologies, particularly regarding contamination and microbiological growth, as well as diminished nutritional and sensory quality during storage. Different methods and technologies have been researched, developed, and implemented to preserve fish quality. Cold storage prevents microbial growth and prolongs fresh fish's

shelf life **(Tavares *et al.*, 2021).** However, cold storage promotes dehydration and increases the oxidation of polyunsaturated fatty acids (PUFA) **(Siddique *et al.*, 2020)**. Vacuum packaging and storage under low oxygen conditions can also boost the effectiveness of preserving the quality of fresh fish **(İzci & Şimşek, 2018; Cao *et al.*, 2020)**. **Rezaabad *et al.* (2017); İzci & Şimşek (2018)** found that vacuum packaging slows the oxidation of fish fillets, especially the oxidation of fat content. Additionally, the introduction of antibacterial and antioxidant active substances can extend the shelf life of fish. A lot of research and development is going into how edible coatings with bioactive substances can be used to keep food fresh.

Chitosan is one of the ingredients explored extensively in creating active packaging to preserve the quality, prolong the shelf life, and enhance the safety of fresh fish products. Because chitosan is biodegradable, non-toxic, edible, biocompatible, has an excellent aesthetic appearance, can block oxygen and physical stress, can inhibit the growth of bacteria, and has antioxidant properties, its use is deemed advantageous **(Dehghani *et al.*, 2018; Socaciu *et al.*, 2018; Barleany *et al.*, 2020; Rahman *et al.*, 2021)**. According to **Inanli *et al.* (2020)**, the DD, MW, concentration, and origin of chitosan influence the effectiveness of chitosan coating in maintaining the quality of fish meat from microbial activity within. Externally, the ability to preserve the quality of fish meat is also determined by the type of fish, pH, temperature, and type of target microbes **(Carrión-Granda *et al.*, 2018)**, as well as the initial quality of fish before treatment **(Pramono *et al.*, 2018)**.

Chitosan coating can inhibit bacterial growth and protein oxidation, prolonging the shelf life of fish meat fillets **(Fan *et al.*, 2009; do Vale *et al.*, 2020).** In the prolong years, many promising studies have been done on how chitosan might work with other bioactive substances to make fresh fish last longer. The use of natural antioxidant and antibacterial ingredients such as carvacrol **(Chaparro-Hernández et al. 2015)**, citrus essential oil **(Li *et al.*, 2019b)**, liquid smoke **(da Silva Santos *et al.*, 2017)**, citric acid and licorice root extract combination **(Qiu *et al.*, 2014)**, ascorbic acid **(Lee *et al.*, 2019)**, buckwheat tartary extract **(Yang *et al.*, 2019)**, cinnamon and tea combination **(Haghighi and Yazdanpanah 2020),** chlorogenic acid **(Cao *et al.*, 2020)**, as well as propolis **(Çoban, 2021)** in chitosan coating can increase the preservation ability of fresh fish fillets. The following is a summary of various natural bioactive substances whose combination with chitosan has been evaluated in terms of their ability to inhibit bacterial growth with TPC parameters Table (1), protein oxidation with TVB-N parameters Table (2), and lipid oxidation with TBAR parameters Table (3).

Table 1. Effect of chitosan and combination with active agents for increasing fish shelf life based on TPC

specification of chitosan	fish species	active agent/ combine	Storage condition	Fish shelf-life (day (log CFU/g))			reference
				without chitosan	with chitosan	chitosan + active agent	
DD = 95% MW = 80-90 kDa	Mackerel (*Pneumatophorus japonicus*)	citrus essential oil	vacuum, -3°C	12 (6,55)	15 (5,40)	15 (5,25)	**(Li et al., 2019a)**
DD* MW*	Tilapia (Oreochromis niloticus)	liquid smoke	non vacuum, 4°C	30 (5,45)	30 (4,55)	30 (3,50)	**(da Silva Santos et al., 2017)**
DD* MW*	Tilapia (*Oreochromis niloticus*)	Tartary buck-wheat extract	non vacuum 0°C	9 (8,90)	15 (7,10)	18 (6,05)	**(Yang et al., 2019)**
DD = 80-95% MW*	Sea bass (*Lateolabrax japonicas*)	citrate acid, licorice root extract	non vacuum, 4°C	12(6,40)	12 (6,00)	12 (5,50) 12 (5,50)	**(Qiu et al., 2014)**
DD* MW*	Sierra (*Scomberomorus sierra*)	tomatoes extract	non vacuum ice storage	10 (7,05)	15 (5,20)	15 (4,80)	**(Ramírez-Guerra et al., 2018)**
DD* MW*	Sea bass (*Lates calcarifer*)	acetic acid	non vacuum 4±1°C	9 (8,70)	12 (6,16)	15 (4,04)	**(Ahmed et al., 2017)**
DD* MW*	Silver carp (*Hypophthalmichthys molitrix*)	nginger extract	non vacuum 4°C	4 (8,95)	8 (8,00)	12 (6,65)	**(Rostamzad et al., 2019)**
DD* MW*	Bighead fish (*Hypophthalmichthys nobilis*)	olive leaf extract	non vacuum 1±4°C	12 (7,75)	12 (7,15)	16 (6,35)	**(Meherpour et al., 2020)**

specification of chitosan	fish species	active agent/ combine	storage condition	Fish shelf-life (day (log CFU/g))			reference
				without chitosan	with chitosan	chitosan + active agent	
DD > 90% MW*	Tilapia (*Oreochromis niloticus*)	carvacrol	non vacuum ice storage	20 (6,2)	20 (4,0)	20 (2,8)	**(Chaparro-Hernández et al., 2015)**
DD 85% MW medium	Rainbow trout (*Oncorhynchus mykiss*)	lactoperoxidase	non vacuum, 4°C	12 (8,47)	16 (6,84)	16 (4,59)	**(Jasour et al., 2015)**
DD 75-85% MW medium	Rainbow trout (*Oncorhynchus mykiss*)	sumac	con vacuum, 4°C	9 (8,40)	12 (6,10)	12 (4,50)	**(Fadıloğlu and Çoban, 2018)**
DD> 90% MW=1.526,5 Da	snakehead fish *(Monopterus albus*)	chlorogenic acid	vacuum, 2-4°C	150 (6,55)	150 (5,25)	150 (5,51)	**(Cao et al., 2020)**
DD* MW medium	Spangled emperor (*Lethrinus nebulosus*)	-	vacuum, film, 4°C *dip-coating*,	6 (9,70)	9 (6,10) 9(6,90)	-	**(Rezaabad et al., 2017)**
DD = ≥75% MW*	Meagre (*Argyrosomus regius*)	-	non vacuum, vacuum, 4°C	< 5 (7,70) 7 (7,25)	- 10(6,90)	-	**(İzci and Şimşek, 2018)**
DD* MW*	sea bass (*Dicentrachus labrax*)	thyme oil	Non vacuum, 4°C	4 (>7,00)	12 (> 7,00)	16 (5,97)	**(Abouel-Yazeed, 2019)**
DD = 75-85% MW = 190-310 kDa	Rainbow trout (*Oncorhynchus mykiss*)	thyme essential oil	Non vacuum, 5±1°C	6 (7,90)	15 (6,72)	15 (6,22)	**(Chamanara et al., 2013)**

* The authors did not mention the characterictic of chitosan.

Table 2. Effect of chitosan and combination with active agents for increasing fish shelf-life based on TVB-N value

specification of chitosan	fish species	active agent/ combine	Storage condition	Fish shelf-life (day (mg%))			reference
				without chitosan	with chitosan	chitosan + active agent	
DD = 95% MW= 80-90 kDa	Mackerel (*Pneumatophorus japonicus*)	citrus essential oil	vacuum, -3°C	3 (>50,00)	12 (38,00)	15 (29,00)	**(Li et al., 2019a)**
DD* MW*	Tilapia (Oreochromis niloticus)	liquid smoke	non vacuum, 4°C	20 (42,00)	30 (9,00)	30 (5,00)	**(da Silva Santos et al., 2017)**
DD* MW*	Tilapia (*Oreochromis niloticus*)	tartary buck-wheat extract	non vacuum 0°C	12 (45,00)	15 (35,00)	18 (25,00)	**(Yang et al., 2019)**
DD = 80-95% MW*	Sea bass (*Lateolabrax japonicas*)	citrate acid, licorice root extract	non vacuum, 4°C	4 (100,20)	8 (60,50)	12 (29,70) 8 (48,00)	**(Qiu et al., 2014)**
DD* MW*	Sea bass (*Lates calcarifer*)	acetic acid	non vacuum 4±1°C	9 (35,00)	12 (32,00)	15 (22,00)	**(Ahmed et al., 2017)**
DD* MW*	Silver carp (*Hypophthalmichthys molitrix*)	nginger extract	non vacuum 4°C	8 (37,50)	12 (29,50)	12 (26,00)	**(Rostamzad et al., 2019)**
DD* MW =30-32 dan 75 kDa	Fish fillets	cinnamon and tea extracts	non vacuum 4-6°C	<5 (61,44)	5 (32,62)	15 (22,42)	**(Haghighi and Yazdanpanah, 2020)**
DD* MW*	Bighead fish (*Hypophthalmichthys nobilis*)	olive leaf extract	non vacuum 1±4°C	16 (29,00)	16 (26,00)	16 (21,00)	**(Meherpour et al., 2020)**

specification of chitosan	fish species	active agent/ combine	Storage condition	Fish shelf-life (day (mg%))			reference
				without chitosan	with chitosan	chitosan + active agent	
DD 85% MW medium	Rainbow trout (*Oncorhynchus mykiss*)	lactoperoxidase	non vacuum, 4°C	8 (37,78)	12 (31,03)	16 (22,07)	**(Jasour et al., 2015)**
DD 75-85% MW medium	Rainbow trout (*Oncorhynchus mykiss*)	sumac	non vacuum, 4°C	6 (38,50)	9 (32,00)	12 (21,50)	**(Fadıloğlu and Çoban, 2018)**
DD* MW medium	Spangled emperor (*Lethrinus nebulosus*)	-	vacuum, film, 4°C *dip-coating*,	3 (41,00)	6 (21,00) 6 (23,00)	-	**(Rezaabad et al., 2017)**
DD = ≥75% MW*	Meagre (*Argyrosomus regius*)	-	non vacuum, vacuum, 4°C	5 (>45,00) 7 (40,00)	- 15 (22,00)	-	**(İzci and Şimşek, 2018)**
DD* MW*	sea bass (*Dicentrachus labrax*)	Thyme oil	non vacuum, 4°C	4 (>30,00)	12 (> 30,00)	16 (27,60)	**(Abouel-Yazeed, 2019)**
DD = 75-85 MW = 190-310 kDa	Rainbow trout (*Oncorhynchus mykiss*)	Thyme essential oil	non vacuum, 5±1°C	9 (37,50)	12 (31,50)	15 (25,00)	**(Chamanara et al., 2013)**

* The authors did not mention the characterictic of chitosan.

Table 3. Effect of chitosan and combination with active agents for increasing fish shelf-life based on TBARS value

specification of chitosan	fish species	active agent/ combine	storage condition	fish shelf-life (day(mg MDA eq/kg))			Reference
				without chitosan	with chitosan	chitosan + active agent	
DD = 95% MW= 80-90 kDa	Mackerel (*Pneumatophorus japonicus*)	citrus essential oil	vacuum, -3°C	3 (>6,50)	12 (5,00)	15 (4,40)	**(Li et al., 2019a)**
DD* MW*	Tilapia (Oreochromis niloticus)	liquid smoke	non vacuum, 4°C	30 (0,31)	30 (0,19)	30 (0,14)	**(da Silva Santos et al., 2017)**
DD* MW*	Tilapia (*Oreochromis niloticus*)	tartary buck-wheat extract	non vacuum 0°C	18 (0,78)	18 (0,50)	18 (0,41)	**(Yang et al., 2019)**
DD = 80-95% MW*	Sea bass (*Lateolabrax japonicas*)	citrate acid, licorice root extract	non vacuum, 4°C	12 (1,65)	12 (1,25)	12 (1,05) 12 (1,00)	**(Qiu et al., 2014)**
DD* MW*	Sea bass (*Lates calcarifer*)	acetic acid	non vacuum 4±1°C	15 (3,00)	15 (1,95)	15(1,50)	**(Ahmed et al., 2017)**
DD* MW*	Silver carp (*Hypophthalmichthys molitrix*)	nginger extract	non vacuum 4°C	12 (1,90)	12 (1,10)	12 (0,70)	**(Rostamzad et al., 2019)**
DD* MW =30-32 dan 75 kDa	Fish fillets	cinnamon and tea extracts	non vacuum 4-6°C	20 (3,71)	20 (2,54)	20 (2,03)	**(Haghighi and Yazdanpanah, 2020)**
DD* MW*	Bighead fish (*Hypophthalmichthys nobilis*)	olive leaf extract	non vacuum 1±4°C	16 (4,45)	16 (3,80)	16 (2,90)	**(Meherpour et al., 2020)**

specification of chitosan	fish species	active agent/ combine	storage condition	fish shelf-life (day(mg MDA eq/kg))			Reference
				without chitosan	with chitosan	chitosan + active agent	
DD 85% MW medium	Rainbow trout (*Oncorhynchus mykiss*)	lactoperoxidase	non vacuum, 4°C	16 (4,78)	16 (4,66)	16 (4,59)	**(Jasour et al., 2015)**
DD 75-85% MW medium	Rainbow trout (*Oncorhynchus mykiss*)	sumac	con vacuum, 4°C	6 (10,50)	9 (6,50)	12 (3,50)	**(Fadıloğlu and Çoban, 2018)**
DD> 90% MW=1.526,5 Da	snakehead fish *(Monopterus albus*)	chlorogenic acid	vacuum, 2-4°C	150 (1,12)	150 (0,57)	150 (0,32)	**(Cao et al., 2020)**
DD* MW medium	Spangled emperor (*Lethrinus nebulosus*)	-	vacuum, film, 4°C	12 (3,30)	12 (1,20)	-	**(Rezaabad et al., 2017)**
DD = ≥75% MW*	Meagre (*Argyrosomus regius*)	-	*dip-coating*, non vacuum,	15 (0,70)	12 (2,10) -	-	**(Izci and Simsek, 2017)**
			vacuum, 4°C	15 (0,45)	15 (0,40)		
DD* MW*	sea bass (*Dicentrachus labrax*)	thyme oil	Non vacuum, 4°C	4 (1,10)	12 (1,28)	16 (1,41)	**(Abouel-Yazeed, 2019)**
DD = 75-85 MW = 190-310 kDa	Rainbow trout (*Oncorhynchus mykiss*)	thyme essential oil	Non vacuum, 5±1°C	15 (0,72)	15 (0,55)	15 (0,39)	**(Chamanara et al., 2013)**

* The authors did not mention the characterictic of chitosan.

Based on the TPC values Table (1), TVB-N values Table (2), and TBARS values Table **(**3), generally, fish meat has a shorter shelf life during cold storage based on the TVB-N and TPC values but more prolonged shelf life when based on the TBARS value. These values mean that oxidation of proteins and bacterial growth has a more inverse effect on fish more than oxidation of lipids. The breakdown of carbohydrate content (glycogen) in fish meat produces lactic acid, which accumulates and can cause protein denaturation. The activity of proteolytic enzymes also leads to protein denaturation. Fish begin to secrete transparent mucus that covers their entire body surface (*hyperemia*), creating an excellent environment for the growth of spoilage bacteria. Proteins will be degraded into amine compounds due to the activity of spoilage bacteria **(Ghaly, 2010)**. The low TBARS value might also result from malondialdehyde's Maillard reaction with free amino acids **(Li *et al.*, 2019b).** According to **Ghaly (2010)** lipid oxidation is the primary source of spoilage and damage to pelagic fish meat, such as mackerel and herring, due to the high oil/fat content in pelagic fish meat. The atmospheric modification provided by vacuum packaging considerably reduces the oxidation of both fat and protein in fish meat during cold storage **(İzci & Şimşek, 2018; Merlo *et al.*, 2019)**.

According to several studies, combining bioactive substances can provide more powerful and effective antioxidant activity **(Cirico & Omaye, 2006)**. However, there are purely additive combinations **(Heo et al. 2007; Olszowy et al. 2019)** or even some that are antagonistic **(Pinelo et al. 2004; Olszowy-Tomczyk 2020)**. In applying the combination of chitosan and bioactive to preserve food products, chitosan functions as a carrier for bioactive substances and is an antibacterial and antioxidant agent **(Coma & Bartkowiak, 2019)**. The combination of chitosan with bioactive substances such as citrus essential oil, ginger extract, tea extract, sumac, thyme essential oil, licorice root extract, olive leaf extract, and tartary buckwheat extract was significantly more effective than chitosan alone in inhibiting bacterial growth and protein oxidation. Hence, extending the shelf life of fish meat **(Jasour *et al.*, 2015; Chaparro-Hernández *et al.*, 2015; Ahmed *et al.*, 2017; Fadıloğlu & Çoban, 2018; Rostamzad *et al.*, 2019; Haghighi & Yazdanpanah, 2020; Meherpour *et al.*, 2020).** Combining chitosan and pomegranate peel extract helps preserve white shrimp's quality **(Yuan *et al.*, 2016)**. Their research demonstrates that chitosan can be coupled with a variety of natural active ingredients to increase the shelf life of fish meat. However, no researchers have investigated whether combining chitosan with various bioactive substances is additive or synergistic.

Deacetylation degree and MW of chitosan influence its ability to inhibit bacterial and oxidant activity. Based on research publications on the use of chitosan to extend the shelf life of fresh fish, there is some information on bacterial growth parameters, TPC Table (1) and protein oxidation, TVB-N Table (2) using chitosan with low (75- 85%) **(Chamanara *et al.*, 2013; İzci & Şimşek, 2018; Fadıloğlu & Çoban, 2018)**, medium (85-90%) **(Qiu *et al.*, 2014)** and high DD (95%) **(Chaparro-Hernández *et al.*, 2015; Li *et al.*, 2019a)**. However, it is impossible to determine from the acquired data whether the DD of chitosan substantially affects its ability to preserve the freshness of fresh fish. In addition to chitosan's DD, other parameters listed in Fig. (4) that affect the shelf life of fresh fish include storage temperature, vacuum packaging treatment, and the various species of fish employed by the researchers. Meanwhile, information about the relationship between chitosan's MW and its ability to preserve the quality of fresh fish is insufficient to conclude. Most studies used chitosan with a medium MW, and the MW and DD of the chitosan were left out of many publications.

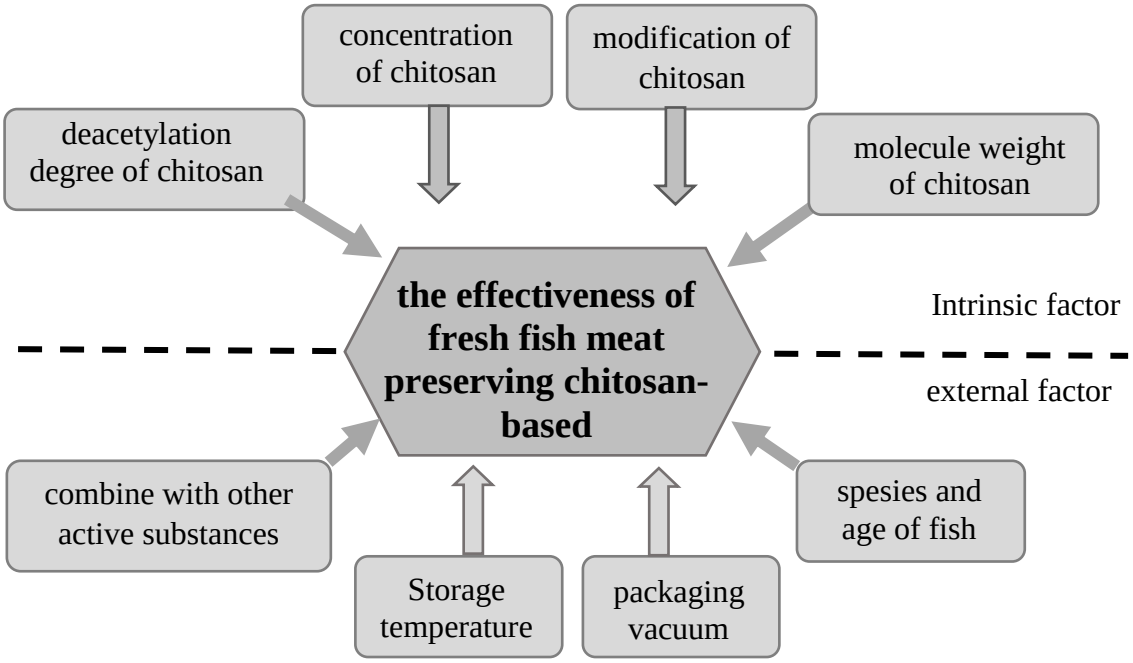

Fig. 4. Different parameters that influence the effectiveness of chitosan-based fresh fish quality protection

CONCLUSION

This review summarizes the potential of chitosan and its combination with other natural active substances to extend the shelf life of fresh fish based on the parameters of the bacterial count, TVB-N value, and TBAR value. This paper suggests that chitosan can preserve the quality and prolong the shelf life of fresh fish. Chitosan's capacity to preserve fresh fish's quality and extend its shelf life is significantly enhanced when combined with other natural active ingredients. Based on this literature review, effective natural active ingredients combined with chitosan to preserve fresh fish include: thyme essential oil, sumac, cinnamon and citrus essential oil. It is unclear, whether this combination is synergistic or merely additive. The correlation between the antibacterial and antioxidant activities of chitosan and chitosan's characteristics has been actively studied. The bioactivity of chitosan is directly proportional to its DD and inversely proportional to its MW. However, there is no direct relationship between the properties of chitosan and its ability to maintain quality and extend the shelf life of fish; in fact, many studies on the use of chitosan in the preservation of fresh fish do not convey the specifications or characteristics of the chitosan used. Based on this, we recommend doing a comprehensive study on the influence of chitosan's DD and MW, as well as the synergistic characteristics of combining chitosan with various bioactive, on its ability to retain the freshness of fresh fish.

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