Salmon Fish Preservation Using Biosynthesized Chitosan By *Aspergillus Niger*

Hero Ahmed Sideeq[1*], Taghreed A. Al-Nashe[2]

1. Department of Food Science & Quality control, College of Agricultural engineering, Sulemani University, Sulemaniyah, Iraq.
2. Department of Food Science & Quality control, College of Agricultural engineering, Sulemani University, Sulemaniyah, Iraq.

*Corresponding author E-mail: hero.sidiq@univsul.edu.iq

Doi:10.29072/basjs.20230111

ARTICLE INFO

Keywords

Chitosan,

Edible coating,

Salmon fish,

TVBN

ABSTRACT

The deacetylated form of chitin found in the cell walls of Aspergillus niger is called chitosan. The United States Food and Drug Administration classifies chitosan, which has many uses, as GRAS. Using three Aspergillus niger isolates, chitosan was produced, and the isolates' capacity to manufacture mycotoxin was determined using polymerase chain reaction (PCR). By using infrared FTIR, manufactured chitosan could be distinguished from commercial chitosan acquired from Sigma. The antibacterial activity of chitosan was investigated using the disk diffusion method and the Minimum Inhibitory Concentration (MIC) assay was conducted against the following bacteria: Salmonella entrica sub entritica ATCC 14028, Clostridium perfrigens ATCC 13124, Escherichia coli, and Streptococcus pneumonia ATCC 49616.It was discovered that Salmonella entrica sub entritica ATCC 14028 was more sensitive than chitosan. Samples of salmon fish were dipped in a 2% chitosan solution, and the samples' shelf life and microbiological quality were assessed. The findings indicated that while pH and moisture loss were tolerable during refrigerated storage, the number of psychrophilic cells was decreased, resulting in a near 12-day storage life.

Received 7 Aug 2022; Received in revised form 16 Sep 2022; Accepted 22 Mar 2023, Published 30 Apr 2023

1. Introduction

Chitosan is copolymers of deacetylation of chitin β-1, 4-Nacetylglucosamine. It produced by alkaline deacetylation of chitin[1-3]. Chitosan have a film-forming ability, it is a natural biodegradable compounds with antimicrobial activity, and generally recognized as safe (GRAS) [4] and environmentally friendly, due to its biocompatibility, no toxicity, antimicrobial activity, and versatile physical and chemical properties [5, 6]. It is commercially produced from crabs and shell of shrimps and in some of microorganisms yeast, mold, and algae [1], fungi *Aspergillus niger* are used as a source for production of chitosan. Antimicrobial activity of Chitosan has been demonstrated against many bacteria and filaments fungi, chitosan have high killing rate against Gram- positive and Gram negative bacteria [7]. Chitosan extract from the cell wall of *Aspergillus niger* [8]. One of the most important microorganisms used in biotechnology is *Aspergillus niger*. Is subject of research and industrial use [9]. Therefore it is important to identify and classify these species based on morphological characteristics, such as colour, size and conidial shape [10] , and Fourier Transforms Infrared spectroscopy FTIR [11]. *A. niger* should be treated similar to other filamentous fungi carefully to avoid the formation of spore dust [12]. Also this group is composed of black-spored Aspergillus species, several of which have a long history of safe use in the fermentation industry. In fact, many *A. niger* enzymes and citric acid are considered GRAS by the United States Food and Drug Administration. Considerable research has been conducted to develop and apply bio based polymers made from a variety of agricultural commodities of food product industrialization. Such biopolymers include starches, cellulose derivatives, chitosan/chitin, gums, proteins (animal or plant-based) and lipids[13, 14], these biopolymers use to preserve foods. Fish flesh is the most perishable food and have short shelf life particularly if not properly handled and stored due to the high post-mortem pH in the flesh, large amount of non-Protein nitrogen like (Ammonia, urea, biure), high content of poly unsaturated fatty acids and presence of autolysis enzyme[15-17]. Salmon is considered to be healthy due to the fish's high protein, high omega-3 fatty acids, and high vitamin D content[18]. The major cause of spoilage of most seafood products are microorganisms. The specific spoilage organisms give rise to the disgusting off-flavours associated with seafood spoilage. This study was amid to keep the quality and the freshness of salmon fish by raped, quart cheep nondestructive and applicable by using edible coating (EC) of 2% chitosan.

2. Materials and Methods

2.1 Isolation and identification of *A.niger*

Three isolates of *Aspergillus niger* were used in this study, tow isolates of *Aspergillus niger* was isolated from the soil by using Solid-state fermentation method [19] and the third isolate obtain it from sigma. The isolates of *A.niger* were grown on (PDA) at 25^{o}C for (96) h. After isolation *A.niger* was purified and identified by the aid of the classification keys of identification according to the work of [20, 21]with the assistance of Doctor Taghreed (College of Agriculture/ Sulemani university), and Spore suspension was prepared from all strain of *A.niger* (*A.niger*1, *A.niger*2, and *A.niger* ATCC from sigma company according to [22]and adjusted to10^{7} spore/ml.

2.2. Determining the ochratoxigenic potential of the identified species

2.2.1. Extraction of Genomic DNA

Fungal genomic DNA was extracted according to the instruction of (BIO Basic Inc, DNA mini press kit) which used to extract DNA of all strain of *A.niger*.

2.2.2 DNA amplification

PCR amplifications were performed in accordance to a procedure as followed by[23]. According to the procedure master mix "BioMix Red" (Bioline, India), 5µl DNA, 20 pmol of identify primers were added and mixed to obtain 50µl final volume of the PCR mix, and used T 100 Thermal cycler to PCR based detection of Ochratoxigenic *A.niger* 1µ of specific primer for detection of Ochratoxin reaction were carried out in volume of containing 5µ (10 pg- 100 ng) of template.

2.2.3 Gel electrophoreses

The DNA of the PCR products were detected on 1% agarose ethidium bromide gel in TAE 1X buffer lanes 1No DNA, lanes 2DNA template of *A.niger* expect size is 690bp according to [24] .

2.3. Determination of growth curve of *Aspegillus.niger*

The growth curves were measured for each isolate by using yeast peptone glucose broth incubated at shaker incubator with 100rpm at 25^{o}C as described by [25].

2.4 Production and Characterization of chitosan

For fungal cultivation 1ml spore suspension (10^{7}spores/ml) added to100 ml of yeast peptone glucose and incubated at 25^{o}C under submerged fermentations condition[26] (100rpm) for 3 days. Mycelia were finely filtrated by using (GF/C glass microfiber; Whatman, Maidstone, UK), and wash twice with D.W then dried at 65^{o}C to constant weight, and dry

fungal mycelia were finely ground, suspended with 1mol NaOH solution (1: 30 w/v) and autoclaved at 121◦C for 30 minutes under 15 psi pressure to partial deacetylation of chitin as well as to hydrolysis of native proteins and nucleic acid. Alkali-insoluble fractions were collected after centrifugation at (12000 g) for 15 min, next washed with distilled water and re-centrifuged to a neutral pH, the chitosan is solubilized using 2% acetic acid (1: 40 w/v) at 95◦C for 8 hours. Slurry centrifuged at (12 000 g) for 15 min, pH of supernatant fluids adjusted with 2mol NaOH solution to (pH 10) , centrifuged at (12 000 g) for 15 min, chitosan precipitate washed with D.W, 95% ethanol(1:20 w/v), and acetone (1:20w/v), respectively, and dried at 60◦C to constant weight, as mentioned by[27]. Chitosan characterized by determination of degree of deacetylation according to[28] by FT-IR spectrophotometer and compared with standard chitosan obtained from sigma company.

2.5. Preparation of chitosan coating solution

As described by [29] 2g of dry weight chitosan was dispersed in 98ml of acetic acid 2 % concentration to obtain 2% (w/w) of chitosan with stirring at 45°C for 2 h.

2.6. Evaluation of antimicrobial effectiveness of coating solutions

2.6.1 Disk diffusion method

The disc diffusion method was done according to[29], by applying deferent concentration of 2% chitosan dipping solution (25, 50, 75, 100 µg/mL), and four species of bacteria that obtain it from sigma (*Streptococcus pneumonia* ATCC 49616, *Clostridium perfrigens* ATCC13124, *Salmonella entrica sub entritica* ATCC 14028, and *E. coli*) were used as indicator microorganisms for the detection of the antimicrobial activity. All strains mentioned above were obtained from sigma and the inhibition diameter of each concentration was measured by (mm) and recorded, all tests were performed in triplicate.

2.6.2 Minimum inhibitory concentration of chitosan

The method that is described by [30] was used to determine Minimum inhibitor concentration of different concentration 2% chitosan (25, 50, 75, 100, 125, 150, 175, 200,and 250 µg/mL). According to [31] McFarland turbidity standard was used to adjust the number of bacteria of 1.5 $x10^8$ CFU/ml. Moreover, the ability of chitosan as inhibiter or cidal against was determined for all bacteria that used in this study as follows: after 24h of inoculated medium that contain different concentration of chitosan by bacteria 0.1 ml was taken from a test tube that doesn't have growth

or turbidity. This is used to inoculate general media (MHA) Mueller Hinton agar then incubate at 37°C for 24h for MIC determination.

2.7 Preserve Salmon fish bay dibbing in 2%chitosan solution at fridge temperature

Fresh salmon fish weight of 2.5 kg was purchase from Sarchnar market then add to cold box until the laboratory, and separated the dorsal part of salmon fish then cut for many slice 5cm^2 sizes, then all pieces dipped in chitosan for three mints, after that leave samples in the room temperature for 90 mints, lastly all samples storage at refrigerator temperature (4°C) [32]. Sample takes after (0, 3, 5, 8, 10, 12, 15,and 17 days) for determination of total volatile basic nitrogen TVBN, pH, Moisture, Microbial test Total count and Psychrotrophes Bactria, and Sensory Evaluation. The determination of Moisture & PH according to [33], the TVBN was described by[34] Microbial test (Total count, Psychrotrophes Bactria) according to[18] .

2.8 Statistical analyses

To data treatment and statistical analyses was used ANOVA to analyses of variance and significant deference, LSD and Fisher test (confidence level, a: 0.05) were performed on the obtained results organize to establish significant differences.

3. Results and Discussion

3.1. Determining the ochratoxigenic potential of the identified species

As shown from the figure 1, the second isolate showed appositive ability for ochratoxin production Since *A. niger* was reported as ochratoxin producer as reported by [35]and excluded from our study while the third and the fourth isolates were non-ochratoxigenic.

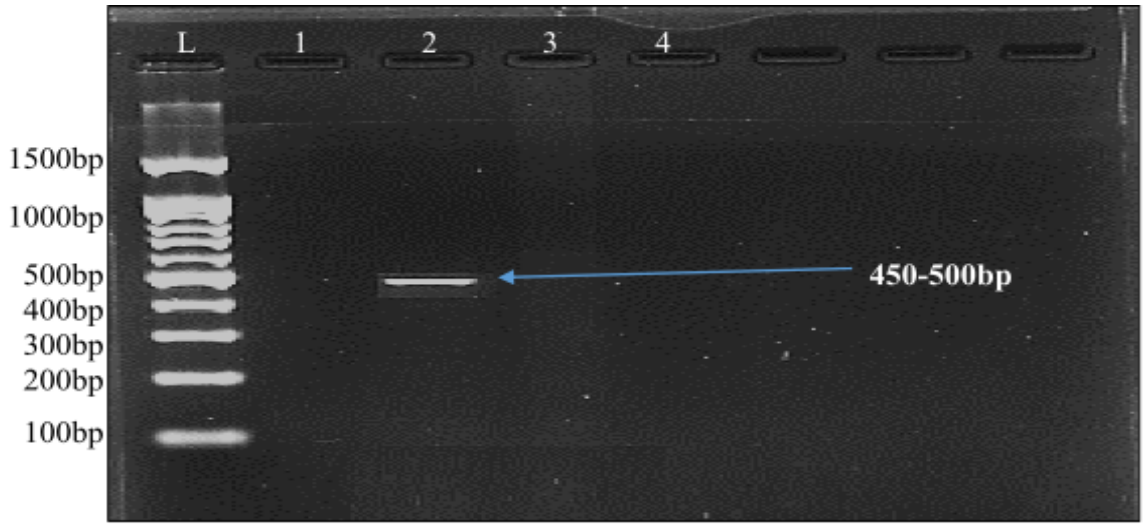

Figure 1: Showing ochratoxin production by PCR method, L: ladder100-1500bp control, lane (1): negative control, lane (2): Ochratoxigenic *A.niger*, lane (3): non-ochratoxigenic *A.niger*, lane (4): non-ochratoxigenic *A.niger* ATCC16404.Used ochratoxin primer: 5´ACTACCGATTGAATGGCTCG3 5´ACGCTTTCAGACAGTGTTCG3´

3.1.1 Determination of growth curve of *Aspegillus.niger*

It is believed that the highest amount of extractable chitosan that can be obtained is at the late exponential growth phase of the fungi. The *A.niger* isolates reached their late logarithmic stage at 72 hours, and after that the measured dry weight of mycelia did not increase, which indicates that the isolates had entered the equilibrium phase. This is also mentioned by [25] that highest yield of chitosan in the first 60 to 72 hours.

3.1.2. Production of chitosan

In this study the *A.niger* isolates were used are (*A.niger* ATCC16404 and *A.niger*1), and table1 present the extracted chitosan form *A.niger* isolates. In table1 the highest yield of chitosan was obtained from (*A.niger* ATCC16404) isolate which produce (4.82gmycelia/1000ml culture), and produce a larger amount of chitosan compare with *A.niger*1. One of the reasons behind selecting *A.niger* ATCC isolates for this study its high productivity of chitosan. As mentioned by[8] the chitosan production from *A.niger* and chitosan content depend on fungal strain, mycelia age, cultivation medium and cultivation condition.

Table.1: Biomass and chitosan obtained from *Aspegillus.niger* isolates

Fungal strain	Weight of Mycelia(g/ml)	Weight of chitosan(g/g)
A.niger ATCC	4.82	1.54
A.niger 1	4.22	1.23

3.2.1 Determination of Degree of Deacetylation (DDA) by using Titration methods

The DDA as shown in the table below, chitosan that was isolated from *A.niger* ATCC and *A.niger1* were 85.85% and 84.38% respectively and both of them were smeller to chitosan standard which

was 92.99%. According to (21), the degree of deacetylation of chitosan ranges from 56% to 99%. According to [36-38] the Degree of Deacetylation of chitosan extracted from *A.niger* was near 85%. Chitosan obtained directly from the fungal cell wall had a higher degree of deacetylation than commercial chitosan from the chemical conversion process (21). Different studies showed that increasing the degree of deacetylation results in an increased antimicrobial activity the antimicrobial activity increases when the degree of deacetylation is higher and the molecular weight is lower. It is important to determine DDA of chitosan because of the electrostatic interaction of chitosan and the negatively charged phospholipids is an important factor in the mode of action. Increasing the degree of deacetylation leads to more free amino groups. As a result more positive charges are located on the polymer, leading to an enhanced electrostatic interaction. Chitosan is hygroscopic in nature and it was reported that the capability of moisture adsorption of chitosan decreased with an increase in deacetylation.

Table2: Determination of Degree of Deacetylation (DDA) by using Titration methods

fungal strains	**DD%**
A.niger ATCC16404	85.85
*A.niger*1	84.38
standard Chitosan	92.99

3.2.2 FT-IR spectrophotometer

The extracted chitosan from fungal cell wall of *A.niger* was analysed by FT-IR spectrophotometer and compared with spectrum of standard chitosan[8]**.** As shown in figures (2,3,4) which is three sample of chitosan(standard chitosan, chitosan that produce from *A.niger1* and chitosan that produce from *A.niger* ATCC), the FTIR analysis showed many peaks that indicate the presence of CH_3 group and N-H deformation of amide group which can be observed clearly in pure chitosan, which decreased dramatically. The peaks obtained from the two samples were found similar to the peaks of standard chitosan witch obtained from standard. This clearly reveals that the extracted fraction from the cell wall of *A.niger* isolates was chitosan[8, 39].

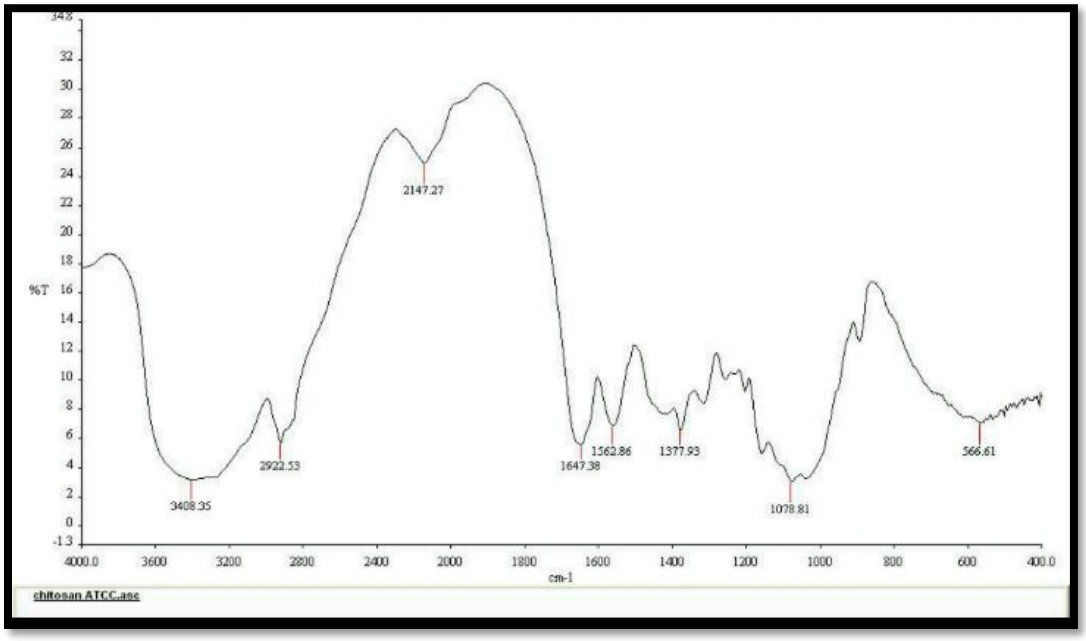

Figure 2: FTIR for chitosan from *A.niger ATCC*

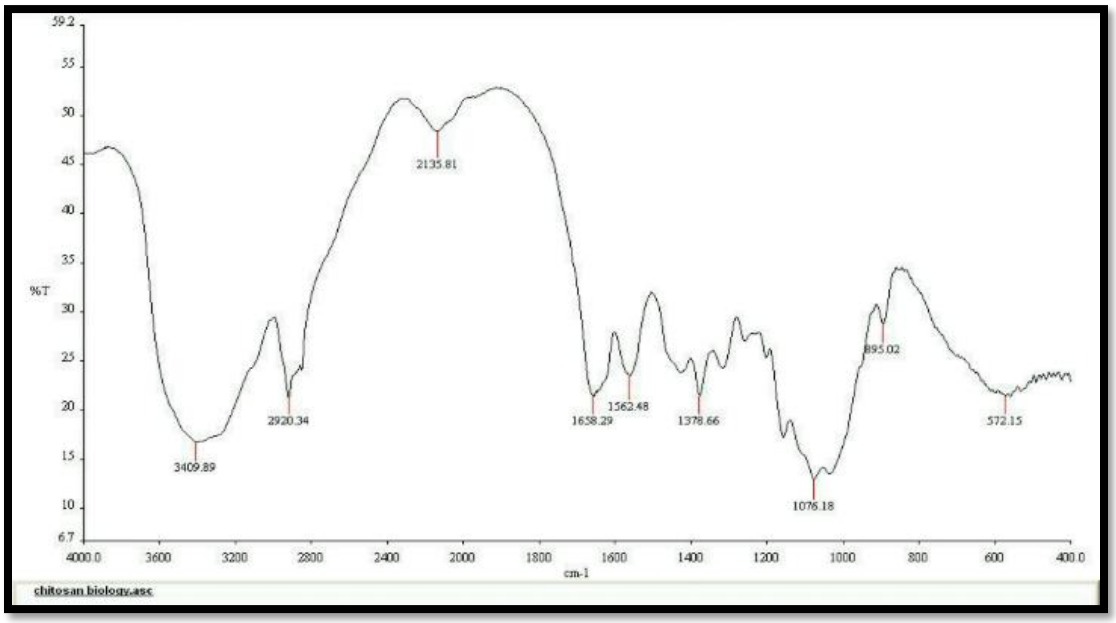

Figure 3: FTIR for chitosan from *A.niger* 1

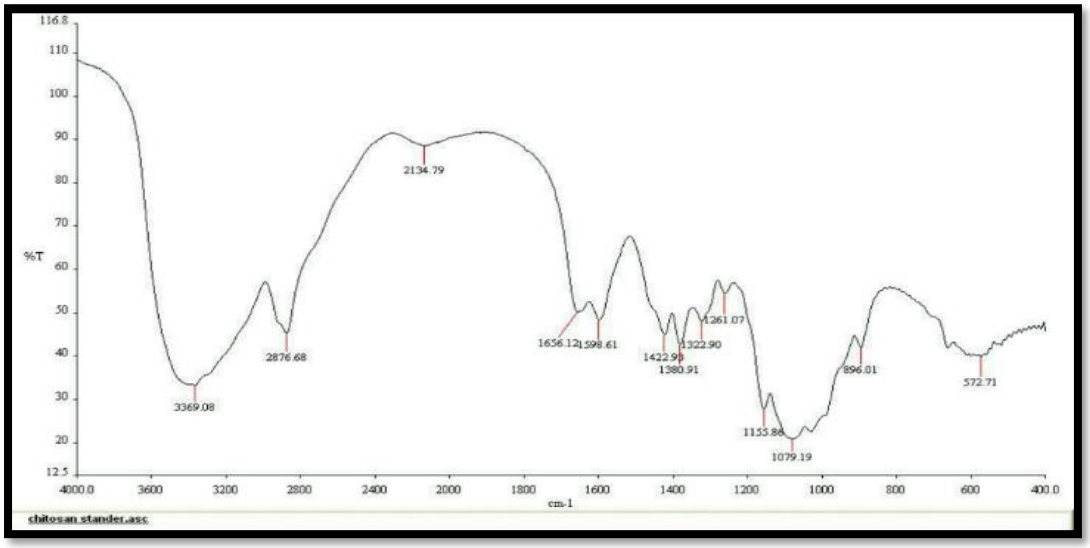

Figure 4: FTIR for standard chitosan

3.3 Evaluation of Antimicrobial Effectiveness of Coating Solution

3.3.1Antibacterial activity of chitosan (Disk diffusion assay) Investigated microorganism were *Streptococcus pneumonia* ATCC 49616, *Clostridium perfrigens* ATCC13124, *Salmonella entrica sub entritica* ATCC 14028, *Escherichia coli*. Disk diffusion zone was used with deference concentration of chitosan (25, 50, 75, 100 µg/mL). As shown from table3 the *E. coli* was more sensitive to 100 µg/mL concentration of 2% chitosan solution than *Salmonella entrica sub entritica*, *Streptococcus pneumonia*, and *Clostridium perfrigens* respectively, this finding is in consist up with the finding of [40], who found that chitosan has strong antimicrobial activity against gram positive and gram negative bacteria such as *Escherichia coli*, also [41] reported that chitosan has antibacterial effect against *Escherichia coli*, and *salmonella*.

Table.3: The results of disk diffusion zone (mm) diameter to chitosan

Conc. µg/mL	*Escherichia coli*	*Salmonella entrica sub entritica*	*Clostridium perfrigens*	*Streptococcus pneumonia*
100	26.667 a a	0.833 a	25.000 a a	22.500 a a
50	18.333 a a	21.667 a a	19.167 ab ab	14.167 ab ab
25	18.333 a a	0.417 a	12.500 c c	17.500 a a
75	16.667 a a	0.000 a	18.333 bc bc	7.500 b b
Pr > F	0.507	0.222	0.010	0.022
Significant	No	No	Yes	Yes

3.3.2 Antibacterial activity of chitosan and antibiotics (Disk diffusion assay)

Figure 5 shows the zones diameter that was measured for all bacterial strains used in this study were *Streptococcus pneumonia* ATCC 49616, *Clostridium* perfrigens ATCC13124, *Salmonella entrica sub entritica* ATCC 14028, and *Escherichia coli*. The result showed that 2% chitosan has a significant different impact (α: 0.05) in terms of inhibition compared to antibiotics (Azithromycin (Az), Amoxicillin (Am), Vancomycin (V), Chloramphenicol (C)[42]. Chitosan diameter zone of inhibition for *Clostridium perfrigens* was more effective agent than (V, Am, Az). However its effect on *Escherichia coli inhibition diameter zone was* less effective agent than Chloramphenicol.

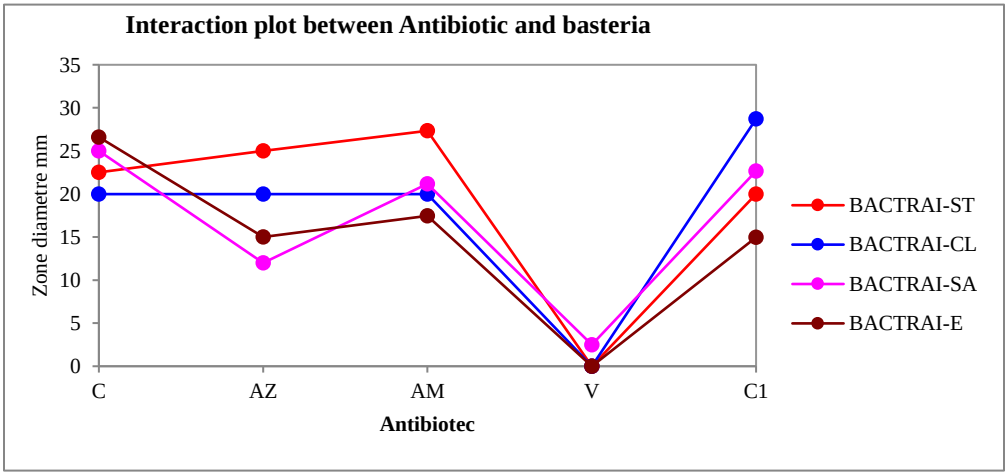

Figure 5: Interaction plot between Antibiotic ((C1) 2% chitosan solution, (V) Vancomycin, (Am) Amoxicillin, (Az) Azithromycin) and bacteria was ((ST) *Streptococcus pneumonia*, (CL) *Clostridium perfrigens*, *(*SA) *Salmonella entrica sub entritica*, (E) *E. coli*).

3.3.3. Minimum inhibiter concentration (MIC) of chitosan in broth medium

As shown from table 4 the 250 µg/mL of 2% chitosan solution was MIC for all bacterial strains that used in this study. *Salmonella entrica sub entritica* was more sensitive than the other bacteria. The 2% of chitosan solution was cidal for *Salmonella entrica sub entritica*, and *Clostridium perfrigens* at concentrations of 100 µg/mL and 175µg/mL respectively. Nevertheless, 2% chitosan was inhibitor for both *Streptococcus pneumonia*, and *E. coli* at concentrations of 200µg/mL and 250µg/ml respectively. The most acceptable mechanism might be the electrostatic interaction between chitosan molecule and microbial cell membrane charges. This leads to the leakage of proteinaceous and other intracellular constituents that finally lead to the death of bacteria, which is also reported by [43, 44].

Table.4: Select MIC of chitosan in broth media.

Tube	**Chitosan Con. µg/mL**	***Streptococcus pneumoni***	***Clostridium* perfrigens**	***Salmonella entrica sub entritica***	***E. coli***
1	25	+	+	+	+
2	50	+	+	+	+
3	75	+	+	+	+
4	100	+	+	-	+
5	125	+	+	-	+
6	150	+	+	-	+
7	175	+	-	-	+

8	200	-	-	-	+
9	250	-	-	-	-
Microbial control		+	+	+	+
Culture media control		-	-	-	-

Positive (+ve) presence of bacterial growth, Negative (-ve) absence of bacterial growth

3.4. Preserve Salmon fish bay dibbing in 2% chitosan stored in refrigera

As Shown in Figure 6 there was a different TVBN value during storage time at 4°C. Salmon sample was acceptable after 15 days of storage at refrigerator temperature while it spoiled after 17 days. On the other hand, controls samples of salmon were spoiled after 8 days of storage time 4°C. As mentioned by[45] the TVBN more than 25 mg N/100g spoilage is detectable.

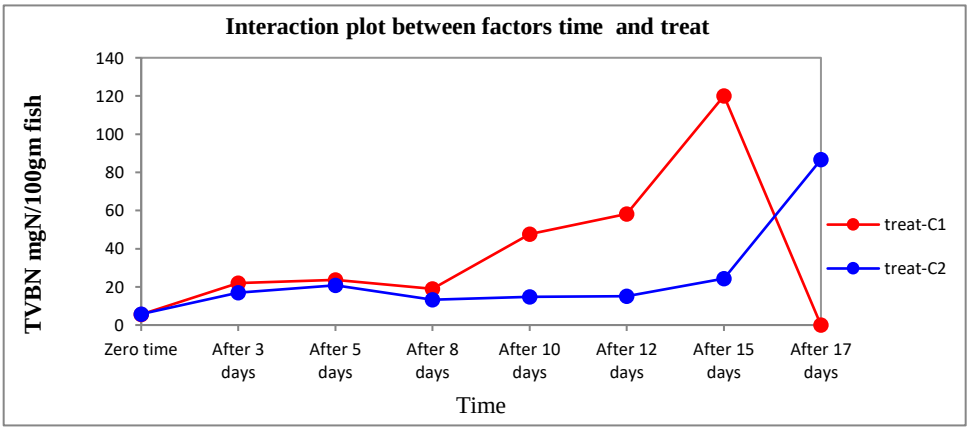

Figure 6: Determine TVBN value of treatments C1: control sample of salmon fish, C2: 2% chitosan during stored time in refrigerator temperature

Figure7 shows the increasing of PH during storage time, because the accumulation of higher concentration of ammonium compounds and other alkaline molecules, as a result of the higher

microbiological and enzymatic activity during chilling condition. It is well-known that the pH value of fish tissue gives valuable information about its hygienic condition. The pH of the samples that coated by chitosan was lower than the control samples during stored period. In the first week the pH was lower because of glycogen in the muscle has been metabolized to lactic acid, but in the end of the first week of storage pH started to increase: reaching a value of pH 7.3 in salmon that coated by chitosan after 15 days. However, for control sample the pH reached 7.7 after 8 days of storage. As mentioned by [29, 46, 47] the pH value must be lower than 7.0–7.5 for the product to be acceptable, In our results samples that were coated by 2% chitosan solution were acceptable for consumption until 15 days of storage in chilling condition.

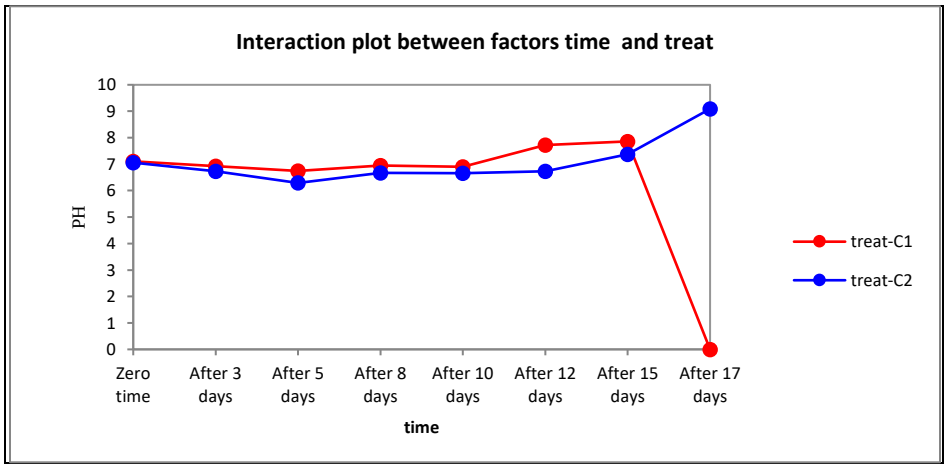

Figure 7: PH content of salmon fish stored at refrigerator temperature, C1: control sample of salmon fish, C2: chitosan coated samples.

The moisture content of salmon fish must be 61.0-70.8% according to [48], in this study moisture was decreased gradually when coated with chitosan. However, in control sample this change was very dramatic. As reported by [49] who verified that coating with chitosan was effective in reducing the moisture loss of salmon by fifty present when compared to the control samples. This means chitosan can protect the quality of salmon sample for longer period. Sensory evaluation of fish gives valuable information of the quality of fish. Time and temperature is main important factor affect spoilage salmon fish. In the result of sensory evaluation (color, texture, odor and Appearance) of salmon fish that were coated by chitosan solution have 18.8/25unit that was better than not coated samples which have 8.45/25 unit.

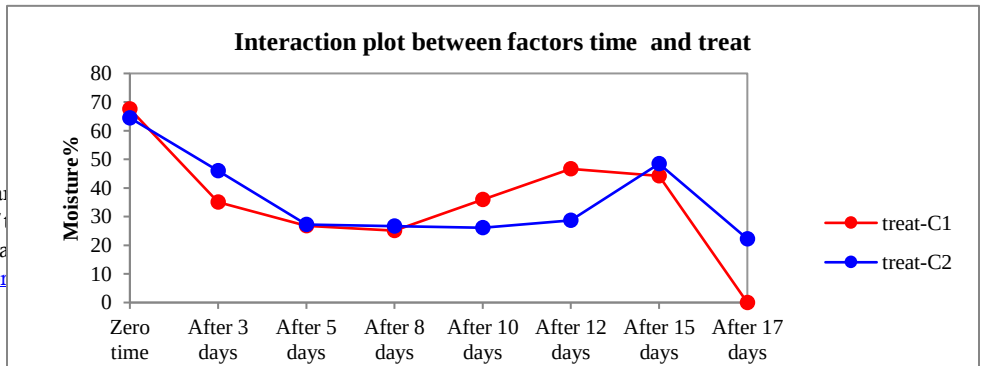

Figure 8: Moisture content of salmon fish storage in refrigerator (4^{o}C), C1 was control sample of salmon fish and C2 was chitosan coated samples.

Table.5: Sensory Evaluation for salmon fish before cooked after 10 days of storage at fridge temperature (4^{o}C)

Sample	Color 5 unit	Texture 5 unit	Odor 5 unit	Appearance 5 unit	General accepter 5 unit	Total 25 unit
Control	2.5	1.65	1.3	1.5	1.5	8.45
Chitosan	3.7	4	3.4	3.7	4	18.8

3.5.1. Microbial test of salmon fish storage in refrigerator (4^{o}C)

As shown from TSable 6 Control the microbial number was uncountable (TNTC) at 8 days of storage, but in chitosan coated samples the microbial numbers was uncountable at 12 days of storage in the chilling condition. Furthermore Microbial spoilage proceeds fast because of the presence of large amounts of low-molecular-weight compounds, high water activity and a high post-mortem pH (> 6) in fish muscles. But chitosan has possibility of obtaining thin films and coatings to cover Salmon fish. In addition, bio films and coatings, by themselves or acting as carriers of foods additives such as antioxidants, antimicrobial have been particularly considered in food preservation because of their ability to extend the shelf life as reported by [29]. Psychrotrophes bacteria important microorganism to spoilage fish in refrigerator temperature, also have greater spoilage activity in low temperature (4^{o}C) had higher demand to amino acid that cause spoilage of salmon flesh and increase in TVBN value, because Salmon is among the ten most consumed species of fish in the world[18], therefore using chitosan to preserve salmon become important way, as reported by [40] that chitosan has antimicrobial activity against Gram-positive and Gram- negative bacteria. As shown in the table7

control samples were spoiled after 3 days and the number of microbial cell was uncountable (TNTC), whereas coated samples by chitosan was uncountable at 10 days of storage in chilling condition.

Table.6: Microbial total count test of salmon fish storage in refrigerator (4°C)

Time	**Control Cfu/ml**			**Chitosan Cfu/ml**		
Zero time	TFTC	TFTC	TFTC	No growth	No growth	No growth
After3 day	$3.1x10^2$	$3.2x10^2$	$3.4x10^2$	$5x10^1$	$5x10^1$	$5x10^1$
After 5 day	$3.8x10^2$	$3.2x10^2$	$3.4x10^2$	$1.7x10^2$	$1.5x10^2$	$1.9x10^2$
After 8 day	TNTC	TNTC	TNTC	$2.2x10^2$	$1.7x10^2$	$2.1x10^2$
After 10 day	TNTC	TNTC	TNTC	$2.7x10^2$	$2.4x10^2$	$3.4x10^2$
After 12 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
After 15 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
After 17 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

Table.7: Microbial Psychrotrophes Bactria test of salmon fish storage in refrigerator 4°C

Time	**Control Cfu/ml**			**Chitosan Cfu/ml**		
Zero time	TFTC	TFTC	TFTC	No growth	No growth	No growth
After3 day	$2.8x10^2$	$2.8x10^2$	$2.4x10^2$	$2.4x10^2$	$2.5x10^2$	$2.5x10^2$
After 5 day	TNTC	TNTC	TNTC	$2.7x10^2$	$2.5x10^2$	$2.9x10^2$
After 8 day	TNTC	TNTC	TNTC	$3.2x10^2$	$3.7x10^2$	$3.3x10^2$
After 10 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
After 12 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
After 15 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
After 17 day	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC

Conclusions

The chitosan coating solution with 2% shown antibacterial activity against all of the tested microorganisms that were employed in this investigation. Additionally, it was an effective coating solution in safeguarding salmon fish for 15 days while stored in the refrigerator. Chitosan has the potential to be used to enhance the shelf life of products.

Referances

[1] N. Nwe, T. Furuike, H. Tamura, Production of fungal chitosan by enzymatic method and applications in plant tissue culture and tissue engineering: 11 years of our progress, present situation and future prospects, Biopolymers, 7 (2010) e162, https://doi.org/10.5772/10261.

[2] A. Verlee, S. Mincke, C.V. Stevens, Recent developments in antibacterial and antifungal chitosan and its derivatives, Carbohydr Polym, 164 (2017) 268-283, https://doi.org/10.1016/j.carbpol.2017.02.001.

[3] S. Ifuku, Chitin and chitosan nanofibers: Preparation and chemical modifications, Molecules, 19 (2014) 18367-18380, https://doi.org/10.3390/molecules191118367.

[4] G.A. Burdock, I.G. Carabin, Generally recognized as safe (GRAS): history and description, Toxicol. Lett., 150 (2004) 3-18, https://doi.org/10.1016/j.toxlet.2003.07.004.

[5] M. Vargas, A. Albors, A. Chiralt, C. González-Martínez, Quality of cold-stored strawberries as affected by chitosan–oleic acid edible coatings, Postharvest Biol. Technol., 41 (2006) 164-171, https://doi.org/10.1016/j.postharvbio.2006.03.016.

[6] D. Campaniello, A. Bevilacqua, M. Sinigaglia, M. Corbo, Chitosan: antimicrobial activity and potential applications for preserving minimally processed strawberries, Food Microbiol., 25 (2008) 992-1000, https://doi.org/10.1016/j.fm.2008.06.009.

[7] M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial properties of chitosan and mode of action: a state of the art review, Int. J. Food Microbiol., 144 (2010) 51-63, https://doi.org/10.1016/j.ijfoodmicro.2010.09.012.

[8] Bhuvaneshwari, S & Velmurugan, Sivasubramanian. (2013). Comparative studies for chitosan yield and chelating ability of Aspergillus niger and Rhizopus oryzae. Indian Journal of Biotechnology. 12. 429-431. http://nopr.niscpr.res.in/handle/123456789/21852.

[9] J.C. Frisvad, T.O. Larsen, U. Thrane, M. Meijer, J. Varga, R.A. Samson, K.F. Nielsen, Fumonisin and ochratoxin production in industrial Aspergillus niger strains, PLoS One, 6 (2011) e23496, https://doi.org/10.1371/journal.pone.0023496

[10] M.A. Kusters-van Someren, R.A. Samson, J. Visser, The use of RFLP analysis in classification of the black Aspergilli: reinterpretation of the Aspergillus niger aggregate, Curr. Genet., 19 (1991) 21-26, https://doi.org/10.1007/BF00362083.

[11] F. Saif, S. Yaseen, A. Alameen, S. Mane, P. Undre, Identification and characterization of Aspergillus species of fruit rot fungi using microscopy, FT-IR, Raman and UV–Vis spectroscopy, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 246 (2021) 119010, https://doi.org/10.1016/j.saa.2020.119010.

[12] E. Schuster, N. Dunn-Coleman, J. Frisvad, P. Van Dijck, On the safety of Aspergillus niger–a review, Appl. Microbiol. Biotechnol., 59 (2002) 426-435, https://doi.org/10.1007/s00253-002-1032-6.

[13] C.N. Cutter, Opportunities for bio-based packaging technologies to improve the quality and safety of fresh and further processed muscle foods, Meat Sci., 74 (2006) 131-142, https://doi.org/10.1016/j.meatsci.2006.04.023.

[14] B.H. Ali, A. Ziada, G. Blunden, Biological effects of gum arabic: a review of some recent research, Food Chem. Toxicol., 47 (2009) 1-8, https://doi.org/10.1016/j.fct.2008.07.001.

[15] J. Tavares, A. Martins, L.G. Fidalgo, V. Lima, R.A. Amaral, C.A. Pinto, A.M. Silva, J.A. Saraiva, Fresh fish degradation and advances in preservation using physical emerging technologies, Foods, 10 (2021) 780, https://doi.org/10.3390/foods10040780.

[16] H.H. Abdel-Naeem, K.I. Sallam, N.M. Malak, Improvement of the microbial quality, antioxidant activity, phenolic and flavonoid contents, and shelf life of smoked herring (Clupea harengus) during frozen storage by using chitosan edible coating, Food Control, 130 (2021) 108317, https://doi.org/10.1016/j.foodcont.2021.108317.

[17] L. Qiu, M. Zhang, B. Bhandari, C. Yang, Shelf life extension of aquatic products by applying nanotechnology: a review, Crit. Rev. Food Sci. Nutr., 62 (2022) 1521-1535, https://doi.org/10.1080/10408398.2020.1844139.

[18] E. Jääskeläinen, L.M. Jakobsen, J. Hultman, N. Eggers, H.C. Bertram, J. Björkroth, Metabolomics and bacterial diversity of packaged yellowfin tuna (Thunnus albacares) and salmon (Salmo salar) show fish species-specific spoilage development during chilled storage, Int. J. Food Microbiol., 293 (2019) 44-52, https://doi.org/10.1016/j.ijfoodmicro.2018.12.021.

[19] Omemu, Adebukunola & Akpan, & Teniola, Olakunle. (2005). Hydrolysis of raw tuber starches by amylase of Aspergillus niger AM07 isolated from the soil. African Journal of Biotechnology

(ISSN: 1684-5315) Vol 4 Num 1. 4. https://www.ajol.info/index.php/ajb/article/view/15046/90965.

[20] R. Samson, i Van Reenen-Hoekstra, ES Introduction to foodborne fungi, Chapter, 6 (1988) 362-368,

[21] R. Siala, A. Kamoun, M. Hajji, L. Abid, N. Gharsallah, M. Nasri, Extracellular acid protease from Aspergillus niger I1: purification and characterization, African Journal of Biotechnology, 8 (2009), https://doi.org/10.5897/AJB09.063.

[22] K. Varalakshmi, B. Kumudini, B. Nandini, J. Solomon, R. Suhas, B. Mahesh, A. Kavitha, Production and Characterization of a-Amylase from Aspergillus niger JGI 24 Isolated in Bangalore, Polish J. Microbiol., 58 (2009) 29-36, https://pubmed.ncbi.nlm.nih.gov/19469283/.

[23] C. Sugita, K. Makimura, K. Uchida, H. Yamaguchi, A. Nagai, PCR identification system for the genus Aspergillus and three major pathogenic species: Aspergillus fumigatus Aspergillus flavus and Aspergillus niger, Med. Mycol., 42 (2004) 433-437, https://doi.org/10.1080/13693780310001656786.

[24] J.D. van Elsas, G.F. Duarte, A. Keijzer-Wolters, E. Smit, Analysis of the dynamics of fungal communities in soil via fungal-specific PCR of soil DNA followed by denaturing gradient gel electrophoresis, J. Microbiol. Methods, 43 (2000) 133-151, https://doi.org/10.1016/S0167-7012(00)00212-8.

[25] K. Nadarajah, J. Kader, M. Mazmira, D. Paul, Production of chitosan by fungi, Pakistan Journal of Biological Sciences, 4 (2001) 263-265, https://dx.doi.org/10.3923/pjbs.2001.263.265.

[26] C.C.C.R. de Carvalho, Fungi in Fermentation and Biotransformation Systems, in: D.-W. Li (Ed.) Biology of Microfungi, Springer, Cham, 2016, 525-541, https://doi.org/10.1007/978-3-319-29137-6_21

[27] A. Shukla, P. Parmar, M. Saraf, Radiation, radionuclides and bacteria: An in-perspective review, J. Environ. Radioact., 180 (2017) 27-35, https://doi.org/10.1016/j.jenvrad.2017.09.013.

[28] R. Czechowska-Biskup, D. Jarosińska, B. Rokita, P. Ulański, J. Rosiak, Determination of degree of deacetylation of chitosan - Comparision of methods, 2012. https://api.semanticscholar.org/CorpusID:51761635

[29] M.B. Vásconez, S.K. Flores, C.A. Campos, J. Alvarado, L.N. Gerschenson, Antimicrobial activity and physical properties of chitosan–tapioca starch based edible films and coatings, Food Res. Int., 42 (2009) 762-769, https://doi.org/10.1016/j.foodres.2009.02.026.

[30] M. Benhabiles, R. Salah, H. Lounici, N. Drouiche, M. Goosen, N. Mameri, Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste, Food hydrocolloids, 29 (2012) 48-56, https://doi.org/10.1016/j.foodhyd.2012.02.013.

[31] A. Zapata, S. Ramirez-Arcos, A comparative study of McFarland turbidity standards and the Densimat photometer to determine bacterial cell density, Curr. Microbiol., 70 (2015) 907-909, https://doi.org/10.1007/s00284-015-0801-2.

[32] M. Moslemi, T. Naderi, Effect of gamma rays on microbial quality of salmon fish during storage in refrigerator at 4° C, Central Asian Journal of Environmental Science and Technology Innovation, 3 (2022) 75-80, https://doi.org/10.22034/CAJESTI.2022.03.02.

[33] C. AACC, Approved methods of the American association of cereal chemists, Methods, 54 (2000) 21, http://dx.doi.org/10.4236/as.2015.65055.

[34] P. MALLE, M. POUMEYROL, A new chemical criterion for the quality control of fish: trimethylamine/total volatile basic nitrogen (%), J. Food Prot., 52 (1989) 419-423, https://doi.org/10.4315/0362-028X-52.6.419.

[35] E.R. Palencia, A.E. Glenn, D.M. Hinton, C.W. Bacon, Constitutive expression of fluorescent protein by Aspergillus var. niger and Aspergillus carbonarius to monitor fungal colonization in maize plants, J. Microbiol. Methods, 94 (2013) 381-389, https://doi.org/10.1016/j.mimet.2013.07.016.

[36] R. Czechowska-Biskup, D. Jarosińska, B. Rokita, P. Ulański, J.M. Rosiak, Determination of degree of deacetylation of chitosan-comparision of methods, Progress on Chemistry and Application of Chitin and its Derivatives, 17 (2012) 5-20, https://api.semanticscholar.org/CorpusID:51761635

[37] B. Ding, H. Gao, J. Song, Y. Li, L. Zhang, X. Cao, M. Xu, J. Cai, Tough and cell-compatible chitosan physical hydrogels for mouse bone mesenchymal stem cells in vitro, ACS Applied Materials & Interfaces, 8 (2016) 19739-19746, https://doi.org/10.1021/acsami.6b05302.

[38] S. Crognale, C. Russo, M. Petruccioli, A. D'annibale, Chitosan Production by Fungi: Current State of Knowledge, Future Opportunities and Constraints, Fermentation, 8 (2022) 76, https://doi.org/10.3390/fermentation8020076.

[39] C. Branca, G. D'Angelo, C. Crupi, K. Khouzami, S. Rifici, G. Ruello, U. Wanderlingh, Role of the OH and NH vibrational groups in polysaccharide-nanocomposite interactions: A FTIR-ATR study on chitosan and chitosan/clay films, Polymer, 99 (2016) 614-622, https://doi.org/10.1016/j.polymer.2016.07.086.

[40] A.A. Mohammed, Production of Chitosan from Aspergillus niger ATCC 16404 and Application as Antibacterial Activity, Journal of Biochemistry, Microbiology and Biotechnology, 6 (2018) 48-50, https://doi.org/10.54987/jobimb.v6i1.391.

[41] I. Younes, S. Sellimi, M. Rinaudo, K. Jellouli, M. Nasri, Influence of acetylation degree and molecular weight of homogeneous chitosans on antibacterial and antifungal activities, Int. J. Food Microbiol., 185 (2014) 57-63, https://doi.org/10.1016/j.ijfoodmicro.2014.04.029.

[42] A. Naskar, K.-s. Kim, Nanomaterials as delivery vehicles and components of new strategies to combat bacterial infections: Advantages and limitations, Microorganisms, 7 (2019) 356, https://doi.org/10.3390/microorganisms7090356.

[43] J.-y. Je, S.-K. Kim, Chitosan derivatives killed bacteria by disrupting the outer and inner membrane, J. Agric. Food Chem., 54 (2006) 6629-6633, https://doi.org/10.1021/jf061310p.

[44] H. Liu, Y. Du, X. Wang, L. Sun, Chitosan kills bacteria through cell membrane damage, Int. J. Food Microbiol., 95 (2004) 147-155, https://doi.org/10.1016/j.ijfoodmicro.2004.01.022.

[45] T.R. Gormley, Survey on fish freshness at retail level, in, University College Dublin. School of Agriculture and Food Science, 2016, http://hdl.handle.net/10197/9509

[46] S. Slattery, 15 Packaging and the Shelf Life of Fish, Food Packaging and Shelf Life, (2010) 279, https://doi.org/10.1201/9781420078459-c15.

[47] H.H. Huss, Quality and quality changes in fresh fish, FAO Rome, documento Tecnico de Pesca (FAO), 1995,https://agris.fao.org/agris-search/search.do?recordID=XF2000387423

[48] J.P. WOLD, T. ISAKSSON, Non-destructive determination of fat and moisture in whole Atlantic salmon by near-infrared diffuse spectroscopy, J. Food Sci., 62 (1997) 734-736, https://doi.org/10.1111/j.1365-2621.1997.tb15446.x.

[49] S. Sathivel, Chitosan and Protein Coatings Affect Yield, Moisture Loss, and Lipid Oxidation of Pink Salmon (Oncorhynchus gorbuscha) Fillets During Frozen Storage, J. Food Sci., 70 (2005) e455-e459, https://doi.org/10.1111/j.1365-2621.2005.tb11514.x.

حفظ اسماك السلمون باستخدام الكايتوسان المصنع حيوياً بواسطة *Aspergillus niger*

هيرو احمد صديق[1] , تغريد عبد وحواح الناشي[2]

1علوم الاغذية والسيطرة النوعية ,الكلية هندسة العلوم الزراعية ,جامعة السليمانية,السليمانية ,العراق

2علوم الاغذية والسيطرة النوعية ,الكلية هندسة العلوم الزراعية ,جامعة السليمانية,السليمانية ,العراق

المستخلص

Aspergillus niger الكايتوسان هو احد اشكال الكايتين المنزوع الاسيتيل من الكايتين الموجود في جدران خلاية تم استخدام ثلاث GRASو يستخدم الكايتوسان في مجموعة واسيعة من التطبيقات و تعتبر ادارة الغذاء والدواء لانتاج الكايتوسان وتم التعوف على قدرة العزلات على انتاج السموم الفطرية بواسيطة تفاعل, *niger.A* عزلات من تضاعف البوليمرات المتسلسل . كان الكايتوسان المصنع مشابها للكايتوسان التجاري الذي تم الحصول علية PCR . تمت دراسة النشاط المضاد للبكتريا للكايتوسانFTIR من سيجما عندما تم تحديده بواسطة الاشعة تحت الحمراء ضد بكتريا MIC بواسطة طريقة انتشار الفرص وتم اجراء اختبار التركيزا المثبط الادنى *Streptococcus pneumonia* ATCC 49616, *Clostridium perfrigens* ATCC 13124, *S entrica* .وجد ان *Salmonella entrica sub entritica* ATCC 14028, and *Escherichia coli* اكثر حساسة تجاه الكايتوسان , تم غمر عينات اسماك السلمون في محلول مكون من 2% من الكايتوسان , وتم قياس التغيرات في الجودة الميكروبية و العمر الافطراضي للعينات واضهرت النتائج ان عددالخلاية النامية في البرودة قد انخفض و ضل الاس الهايدروجيني و فقدان الرطوبة مقبولين طوال فترة التخزين المبرد,مما ادى الى اطالة عمر التمخزينالى ما يقرب من 12 يوما.