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Production of Chitosan and Fucoidan Extracted from Brown Seaweed *Sargassum latifolium* Composite Films with Hydrolyzed Fish Protein from Carassius (*Carassius carassius*)

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ABSTRACT

The aim of this study was to investigate the properties of edible composite films made from chitosan and fucoidan combined with hydrolyzed fish proteins from Carassius. In this context, hydrolyzed fish protein was produced using the enzyme Alcalase, and fucoidan was extracted from the brown seaweed *Sargassum latifolium*. Subsequently, five types of edible films were prepared, including nano chitosan and chitosan-fucoidan with varying concentrations of hydrolyzed protein (0.0, 0.5, and 1.0 percent), and their properties were examined. The results indicated that the hydrolyzed protein had a high protein content and degree of hydrolysis. Additionally, the yield of fucoidan extraction was 5.89%, with total carbohydrates at 59.2%, protein at 21.9%, sulfate at 21.78%, and uronic acid at 8.85%. Mechanical tests showed that increasing protein concentration resulted in a decrease in the tensile strength of the films. Furthermore, physical tests revealed that higher protein concentrations led to increased water vapor permeability and moisture content of the films ($p < 0.05$). The hydrolyzed fish protein exhibited significant activity in scavenging DPPH free radicals, with an increase in concentration positively impacting this parameter ($p < 0.05$). Moreover, these films demonstrated high antimicrobial properties against pathogenic bacteria, with superior antimicrobial activity against *Staphylococcus aureus* compared to *Escherichia coli*. Overall, the findings of this study suggest that chitosan-fucoidan composite edible films incorporating hydrolyzed proteins can serve as a suitable option for enhancing the quality and shelf life of food products. These films not only possess improved physical and mechanical properties but also exhibit antioxidant and antimicrobial activities that can contribute to increased food safety and quality.

1- Introduction

The challenge for food manufacturers is to maintain product quality during storage. Biodegradable films and coatings, usually made from biopolymers, can extend the shelf life of foods.[1]. Active edible films extend the shelf life of food products by controlling the release of bioactive compounds.[2]. Seaweed, especially the brown kind*Sargassum latifolium*They contain valuable polysaccharides that are used in the food and pharmaceutical industries.[3، 4].Fucoidan, one of the most important polysaccharides found in brown algae, is known as a bioactive compound.[5]. The biological properties of fucoidan, including its ability to form antimicrobial films and biodegradability, make it a suitable option for use in the food and health industries.[6، 7]. Since the individual composition of each material in the film may have weaknesses, combining different materials can help improve the properties of edible films.[5، 6].Chitosan helps increase the shelf life and quality of food under anaerobic conditions, and its antimicrobial and antioxidant properties are enhanced by adding ingredients such as hydrolyzed proteins.[8]. Also, the structure of chitosan with positive functional groups makes it a biodegradable and hygienic option.[1، 9]. The use of chitosan coatings can prevent enzymatic browning, retain water, maintain natural flavor, and improve the texture quality and color stability of foods.[10]. Combining fucoidan and chitosan can help improve the properties of edible films and increase the quality of food products.[7، 10].

Carp fish*Carassius carassius*), fromIt is a member of the cyprinid family and has little commercial value due to its small size and poor taste. It is known as a source of protein in some countries and is considered a nuisance fish in warm-water fish farming ponds due to food competition with carps. Given its low price and high production, processing it into value-added products has attracted attention. [11]. Extensive research has been conducted on natural antioxidants and the antioxidant capacity of peptides derived from hydrolyzed proteins such as soy, wheat, and milk [12, 13]. Enzymatic hydrolysis can help recover bioactive peptides with low molecular weight that are easily absorbed by the body and play important roles at the cellular level [14, 15]. These peptides are important because of their antioxidant properties and ability to scavenge free radicals. [12، 13، 16]. So far, no research has been conducted on the effect of hydrolyzed crucian carp protein on chitosan-fucoidan composite films. Therefore, the aim of this study is to investigate the physical, mechanical, antioxidant, and antimicrobial properties of a composite film containing chitosan and fucoidan along with hydrolyzed crucian carp protein.

2- Materials and methods

2-1-Raw materials

Algae*Sargassum latifolium* , native to marine areas, was obtained from the Risher region located in Bushehr province. The macroalgae were cleaned and then washed several times with fresh water andAvon(Memmert Model 500 UFB, Germany) were dried at 38°C.Then, grind it into powder using a grinder (Bosch model TSM6A011W, Germany) andThe plastic bags were stored at 20°C. Carp fish with an average weight of 100 g were obtained from Sari fish market, Mazandaran, Iran. Alcalase enzyme (with a specific activity of 2.4 Anson units/g and a density of 1.18 g/ml) is an endoproteinase obtained from bacteria.*Bacillus licheniformis*The enzyme was purchased from Novozyme, Denmark, and stored at 4°C until the experiment. Ethanol buffer, Tris, and hydrochloric acid were from Merck, and DPPH reagent was from Sigma, and all chemicals used in the experiment were of laboratory grade.

2-2- Preparation of hydrolyzed protein from crucian carp:

After skinning, head and tail removal, and removal of intestines and viscera, the crucian carp was washed with water and then pounded three times byMeat grinder(Electrocar Steel Model EC16, Iran) was ground (5 mm pore diameter) and then stored in zippered plastic bags in a freezer at -20°C until the time of testing. Frozen ground crucian carp meat was placed in the refrigerator overnight to defrost, then weighed into flasks and placed in a 100 ml beaker to inactivate endogenous enzymes.Water bath (Memmert model WNE45) , Germany)at 85°C for 20 minutes. The heated samples were suspended in Tris-chloric acid buffer in a weight-volume ratio of 1:2 to a uniform pH suitable for

alkalase enzyme activity (pH=8.5). The hydrolysis reaction was carried out at 50°C with an enzyme concentration of 1%. All reactions were carried out in 100 ml flasks in Shaker water bathand was carried out at a constant speed of 200 rpm and at the desired temperature. At the end (90 minutes), in order to ensure enzyme inactivation, the enzymatic reaction was completed by heating the suspension at 85°C for 10 minutes and the hydrolyzed compound was cooled in an ice bath and thenRefrigerated centrifuge(Model HB800, Iran) at 8000g at 10°C for 20 minutes to collect the supernatant. The hydrolyzed protein was stored in a freezer and then analyzed usingFreeze dryer (Alfa-Christ, Germany)It came in powder form [15].

2-3- Degree of hydrolysis

The degree of hydrolysis was calculated based on the amount of α-amino acids in the sample protein. [15].

2.4- Amino acid composition

In order to obtain the amino acid composition of hydrolyzed protein powder, the protein sample was first completely hydrolyzed for 24 hours at 110°C using 6M hydrochloric acid. Then, the amino acids present were derivatized by adding phenyl isothiocyanate (PITC). The total amino acids were measured using a Smart line HPLC (made in Germany) equipped with a C18 column and a fluorescent detector (RF-530). [12].

2-5- Extraction of fucoidan and examination of its properties

20 g of dried seaweed powder was twice stirred in 400 ml of pure ethanol for 3 h at room temperature to remove unwanted compounds. Then, the seaweed biomass was washed with distilled water and dried in a vacuum oven at 45 °C to reach constant weight. In order to extract fucoidan by the usual extraction method, the seaweed biomass was treated with a solvent:algae ratio of 20 ml/g for 160 min at 45 °C (heater stirrer). The resulting mixture was cooled in an ice bath and its pH was increased to 7 by titration with 2 M sodium hydroxide and neutralized. Then, the precipitated phase was separated by vacuum filtration. The supernatant was treated with two volumes of pure ethanol at 4 °C overnight to precipitate fucoidan. The resulting precipitate was separated by centrifugation at 7000 g for 15 min at 4 °C and collected for purification. The isolated fucoidan was washed twice with 70% ethanol and finally dried at 45°C in an oven under vacuum.[17].

The extraction efficiency was calculated by the following formula[18].

100×Dry weight of algae (grams)/Dry weight of fucoidan (grams)=Yield (percentage)

The amount of fucoidan sulfate was measured after its hydrolysis in 0.5 M hydrochloric acid at 105 °C for (5 hours) according to the method mentioned by Lee et al. (2012) and using barium chloride (BaCl) and potassium sulfate (K2SO4) as standards.[19].

Protein levels were measured according to the Lowry method using a protein assay kit.[20].

Total carbohydrate was measured according to the method mentioned by Gorji et al. (2022) using the androgynous method and measuring the absorbance at 630 nm.[20].

The uronic acid level will be determined according to the method described by Filisetti-Cozzi and Carpita (1991) using glucuronic acid as a standard.[21].

2-6- Preparation of chitosan-fucoidan film

To prepare the composite film, chitosan (at a concentration of 2%) and fucoidan (at a concentration of 1%) were added to the mixture along with different concentrations of hydrolyzed protein (0.5 and 1%). These compounds were completely dissolved in 200 ml of distilled water at a temperature of 70 °C for 45 minutes with stirring. By casting method[1]To obtain the films, 50 ml of each solution was poured into a 12 cm Petri dish and dried at room temperature for 48 hours. Then, the films were separated and stored in Stomaker bags.[8].

7-2-Measurement of physical properties of films

1-7-2-Measurement of film thickness

A micrometer with an accuracy of 0.01 mm was used to measure the thickness. The thickness was measured at 5 points on the film and the average value was reported [22].

2-7-2-Measuring the moisture content of films

1-Casting

Film samples of a certain weight were placed in glass plates that had been previously weighed and brought to a constant weight (W1). They were then dried in an oven at 105°C for 24 hours. The sample along with the plate was removed after this period and, after cooling in a desiccator, reweighed (W2). The moisture content of the films was calculated based on the wet weight from equation 1.[23].

Relationship 1

100×W1/(W2-W1) = Humidity percentage

3-7-2-Water vapor permeability

In order to measure the permeability of the films to water vapor (ASTM E 96-02), first 10 ml of distilled water was poured into the permeability measurement cells and then the glass cells, whose surfaces were sealed by the film and grease, were placed in a desiccator containing silica gel. Water at a temperature of 25 °C creates 100% humidity. The difference in humidity on the two sides of the film at a temperature of 25 °C creates a vapor pressure difference equal to 2.337 × 103 Pascal. The changes in the weight of the cells over time were measured using a digital balance with an accuracy of 0.0001 g. The water vapor transmission rate in (g)-m-s was equal to the slope of the resulting lines divided by the cell area and was obtained from equation 2 [19]. The area of the cells was 0.00287 m2. By multiplying the water vapor transmission rate (WVTR) by the film thickness (L) and dividing it by the pressure difference on both sides of the film (AP), the water vapor permeability (WVP) (10^{-11} $gs^{-1}m^{-1}Pa$) was obtained[23].

Relationship 2

Cell area (m) / line slope (g/s) = water vapor transmission rate ($g^{1-}Seconds^{2-}meters$)

8-2-Mechanical properties of films

Mechanical tests of the films were performed according to the modified ASTM D0882-02 method. The films were cut into 761 cm pieces and conditioned under conditions of 50% relative humidity and 25°C. Their thickness was measured at 5 points and their average thickness was determined. The mechanical properties of the films (tensile strength (MPa)) were determined usingMeasuring from the Instron deviceIn the Instron device, the distance between the two jaws was 50 mm, the speed of movement of the upper jaw was 50 mm/min, and the lower jaw was fixed [23].

2.9- Antioxidant activity of films

The antioxidant activity of the film was measured using the DPPH free radical scavenging assay [24]. For this purpose, first 25 mg of the film was gently mixed in 3 ml of distilled water. Then, 2.8 ml of this solution was added to test tubes.A solution containing 0.2 ml of 1 mM DPPH solution in methanol was added and kept at room temperature for 30 minutes. The optical absorbance of the samples and the control sample was measured at a wavelength of 517 nm using a T80 spectrophotometer made in England. ReductionThe optical absorption compared to the control indicated the ability of the compounds in the film to scavenge DPPH free radicals. Finally, the percentage of DPPH free radical scavenging activity was calculated using equation 3.

Relationship 3:

[100]×(Control absorbance / Sample absorbance – Control absorbance)- 1 [= DPPH free radical scavenging percentage

2-10-Determination of antimicrobial activity of films

Bacterial cultures*Staphylococcus aureus*(PTCC 1189) and*Escherichia coli*(PTCC 1399) was obtained from the microbiological collection of the University of Tehran. Using a sterile loop, a quantity of each bacterium was removed from sterile ampoules and added to 10 ml of BHI Broth culture medium. The inoculated culture medium was incubated for 24 hours at 37°C. After incubation, the bacteria were cultured on nutrient agar plates using a sterile loop. The cultured plates were incubated for 24 hours at 37°C. 3 to 5 colonies were isolated and homogenously transferred to tubes containing 5 ml of physiological serum using a sterile swab. The turbidity (light absorption) of the bacterial suspensions was measured at a wavelength of 625 nm using a spectrophotometer. The suspensions were diluted to a concentration equal to half McFarland, which is equivalent to about10^8 colonies per ml. Using a sterile swab, the bacterial suspensions were spread evenly over the surface of the culture media. Round film discs with a diameter of 6 mm were cut using a round knife. The film discs were placed at appropriate intervals on the plates

coated with bacteria and the plates were incubated for 24 hours at 37°C. After incubation, the diameter of the clear halos around the film discs was measured in millimeters and reported. This method was used to investigate the antimicrobial activity of edible films using the agar diffusion method [25].

11-2- Statistical evaluation

The experiments were conducted in three replications in a completely randomized design. Data analysis was performed using SPSS 18 software. Comparison of means was performed using Duncan's test (One way Anova) with a probability level of 5%. Graphs were drawn using Microsoft Excel 2013 software.

3- Results and discussion

3-1-Degree of hydrolysis

Controlling the rate of hydrolysis progress is of great importance in the hydrolysis process, because the properties of hydrolyzed protein, including free amino acids, solubility, and antioxidant properties, depend on the degree of hydrolysis. The results showed (Table 1) that the hydrolysis time has a significant effect on the degree of hydrolysis, with the highest degree of hydrolysis recorded at 90 min and the lowest at 30 min. Also, the rate of hydrolysis increased more rapidly up to 60 min ($P<0.05$). In the early stages of hydrolysis, the chemical bonds of proteins are more accessible and water can easily break these bonds. In addition, increasing the concentration of amino acids and reducing the molecular weight of peptides in the early stages improves solubility, and enzyme activity also reaches its peak at this stage. These factors cause the intensity of hydrolysis to be higher early in the process. Similar results have been reported for the degree of hydrolysis of hydrolyzed fish protein using commercial enzymes. According to studies, as time increases, the degree of hydrolysis also increases, and the rate of increase is higher in the early stages of the hydrolysis process [11, 12, 13, 15, 16, 26].

3.2- Protein amount

The protein content of crucian carp and its hydrolyzed protein powder is 0.95, respectively.±17.01% and in the range of 52.55-83.23% (Table 1). The results show that hydrolyzed crucian carp protein contains a significantly higher amount of protein than the original fish. This has been confirmed in previous studies [15, 16, 26]. This increase in protein content is due to the removal of moisture from the samples and the separation of non-protein compounds in the hydrolysis and centrifugation process. In fact, the hydrolysis process causes proteins to be converted into more absorbable forms that are richer than crude proteins, and these properties can have wider applications in the food and nutritional industries. Mehregan Nikoo et al. (2014) reported the initial protein content of crucian carp to be 16.43% and 79.13% after the hydrolysis process, which are almost consistent with the present study [11]. This similarity indicates the stability of the hydrolysis process in increasing the amount of usable protein in crucian carp and emphasizes the effectiveness of this process in enhancing the nutritional value of this protein source.

Table 1: Degree of hydrolysis and protein content of crucian carp protein hydrolysates at different hydrolysis

Hydrolysis time (min)	Degree of hydrolysis (%)	Protein content (%)
30	13.99±0.78[c]	52.55±0.99[c]
60	19.95±0.88[b]	71.39±1.16[b]
90	28.32±1.11[a]	83.23±2.29[a]

[a] Values represent means ± SE (n = 3).
[b] Values in same columns with different lower letter are significantly different at $P < 0.05$.

3-3- Amino acid composition

Fish protein hydrolysates have been increasingly considered as valuable sources of absorbable peptides and amino acids, as these products have several properties, including antioxidant, anti-inflammatory, anticancer, and antibacterial effects [16, 27]. According to studies, essential amino acids are of great importance for the growth and development of the human body. In this study (Table 2), all essential amino acids

recommended by WHO/FAO (1990) for daily intake of adults were present within the permissible limits. Glutamic acid, which is known as one of the key amino acids in fish, plays important physiological roles in the body [28]. In this study, the highest amount of non-essential amino acid was related to glutamic acid with a percentage of 78.9%. Also, lysine was identified as an important essential amino acid with a content of 13.05% in this fish and accounted for the highest amount of essential amino acids, indicating the high potential of this fish as a source of quality protein [27]. The total hydrophobic amino acids in hydrolyzed proteins reached 41.25%. The presence of these amino acids at optimal levels indicates their high nutritional value. Hydrophobic amino acids, such as leucine, isoleucine, phenylalanine and valine, play a vital role in the function of proteins due to their ability to maintain protein structure and create appropriate physical and chemical properties. These amino acids also act as precursors for the synthesis of bioactive compounds and can be effective in improving antioxidant activities, anti-inflammatory properties and regulating blood sugar and blood pressure [29]. According to Luo et al. (2022), crucian carp protein has high potential for use in the food industry and improving nutritional health due to its appropriate amino acid composition [27]. These characteristics make crucian carp a suitable option for meeting protein needs and can be used in various nutritional programs.

Table 2: The amino acid composition crucian carp protein hydrolysates (g 100 g^{-1}) (30 min)

Amino acid(g 100 g^{-1})	Alcalase	FAO/ WHO, 1990
Histidine [a]	4.77	
Isoleucine [a]	5.88	2.8
Leucine[a]	11.21	6.6
Lysine[a]	13.05	5.8
Methionine[a]	3.24	-
Phenyl alanine[a]	6.44	6.3
Tyrosine	4.24	1.1
Threonine[a]	5.28	3.4
Arginine	8.58	-
Valine[a]	4.55	3.5
Aspartic acid	7.98	-
Glycine	3.87	-
Proline	3.48	-
Serine	3.58	-
Alanine	4.21	-
Glutamic acid	9.78	-
Total amino acid	98.14	-

LET[b]	41.25	-

a Essential amino acids
b Total hydrophobic amino acids (alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, proline, methionine and cysteine)

3.4 Review of fucoidan tests

Results obtained from tests of fucoidan extracted from brown algae*Sargassum latifolium* In this study, the extraction efficiency is as follows:89.5±0.35%, total carbohydrate equal to 59.2±31.18%, protein equal to 9.21±0.58%, sulfate equal to 21.78±21.18%and uronic acid85.8±0.18 percent,In the study by Gorgij et al. (2022), the extraction efficiency of fucoidan from brown algae was reported to be 23.7%, total carbohydrates 36.51%, protein 64.5%, sulfate 11.57%, and uronic acid 22.9%.[20]Also, in another study, Lee et al. (2012) reported the extraction efficiency and the amounts of total carbohydrates, sulfate, uronic acid, and protein of fucoidan extracted from brown algae.*Ecklonia Cava* They announced 1.8, 51.8, 1.20, 3.11, and 7.8 percent, respectively.[19]In general, fucoidans isolated from algae are usually complex compounds, and the percentage of their components varies depending on the species, extraction method, and experimental conditions.[20، 30].

3-5-Investigating the physical properties of films

According to the results (Figure 1), the highest moisture content was observed in the chitosan film. The addition of fucoidan reduced the moisture content of the film (P<0.05). The decrease in moisture content of the chitosan film after the addition of fucoidan is mainly related to the creation of a tighter network structure and the hydrophobic property of fucoidan. These features reduce water absorption and limit water contact with the film surface. Similar results were obtained byGomaa et al. (2018), they also reported that the addition of fucoidan to chitosan-alginate films after reducing the moisture content of the film [31]. AlsoPouralkhas et al. (2023) also reported that adding fucoidan to alginate film reduces the moisture content of alginate film [7].However, the addition of hydrolyzed protein significantly increased the moisture content. Hydrolyzed proteins have a significant effect on the moisture content of food products due to their hydrophilic properties. These proteins have the ability to absorb and retain more water than non-hydrolyzed proteins and can modulate moisture fluctuations. The addition of these compounds to food products improves texture, enhances flavor, and maintains quality and shelf life [16]..

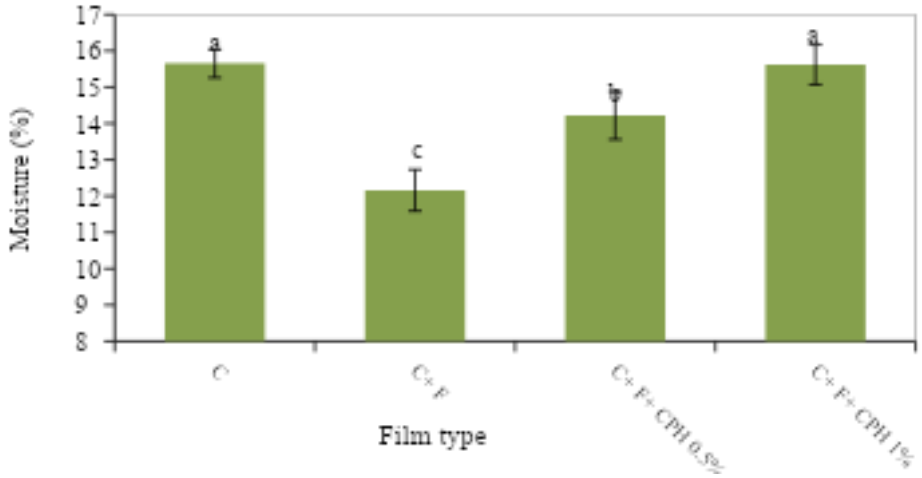

Fig 1. Moisture content of composite film along with crucian carp protein hydrolysates

The results show (Figure 2) that the lowest thickness values were observed in chitosan and fucoidan films (P<0.05). In fact, the addition of fucoidan had no significant effect on the film thickness values (P>0.05), indicating a good compatibility between the film components. These results are consistent with the findings of Samani et al. (2023) who reported that the addition of fucoidan to chitosan-agar films had no significant effect on the film thickness [10]..On the other hand, by adding hydrolyzed protein to the film, the thickness increased and

the highest thickness was observed in the films of chitosan + fucoidan + 1% hydrolyzed protein (P<0.05). Similar results have been reported by other researchers; for example,Abugoch et al. (2010) reported that as the ratio of quinoa protein to chitosan increases, the thickness of the chitosan film also increases [31]. Also,Oliveiraet al. (2019) observed similar results in their studies on hydrolyzed cottonseed protein in alginate films [32]. In general, previous studies show that the difference in film thickness depends on the preparation method and the amount of additive.

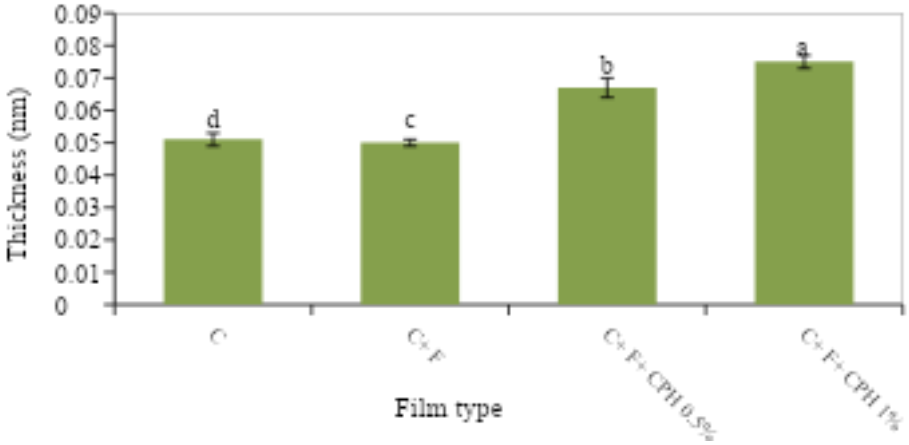

Fig 2. Thickness o f composite film along with crucian carp protein hydrolysates

According to the results (Figure 3), the addition of fucoidan to the chitosan film resulted in a decrease in the permeability values of the films (P<0.05). This decrease in permeability is due to the addition of a crosslinking agent that helps to increase the interactions between the chitosan and fucoidan polymers and create a barrier against water vapor. In general, the addition of fucoidan and the crosslinking agent is associated with the formation of hydrogen bonds, which probably reduces the water vapor permeability of the films [10]. These results are consistent with the findings of Samani et al. (2023) who reported that the addition of fucoidan to the chitosan-agar film caused a decrease in the water vapor permeability of the films.WVPThe film was [10]. By adding hydrolyzed protein to the film, the permeability increased, so that the highestWVPIn the films of chitosan + fucoidan + hydrolyzed protein 1% was observed (P<0.05). The reason for this is related to the plasticizing effect of hydrolyzed proteins with low molecular weight. The presence of these proteins leads to an increase in hydrophilic groups in the film structure, which, as a result, a greater number of water molecules are placed in the film, which leads to an increase in water vapor permeability (WVP) film. Also, increasing the thickness of the hydrolyzed protein layers also has a significant effect on the WVP values [32, 33]. Similar results were obtained byOliveiraet al. (2019) in their studies on hydrolyzed cottonseed protein in alginate film andGhasemi et al. (2021) observed in carboxymethylcellulose film containing hydrolyzed silver carp skeleton protein [10]. These findings indicate the importance of hydrolyzed proteins in improving the properties of edible films and their impact on packaging performance.

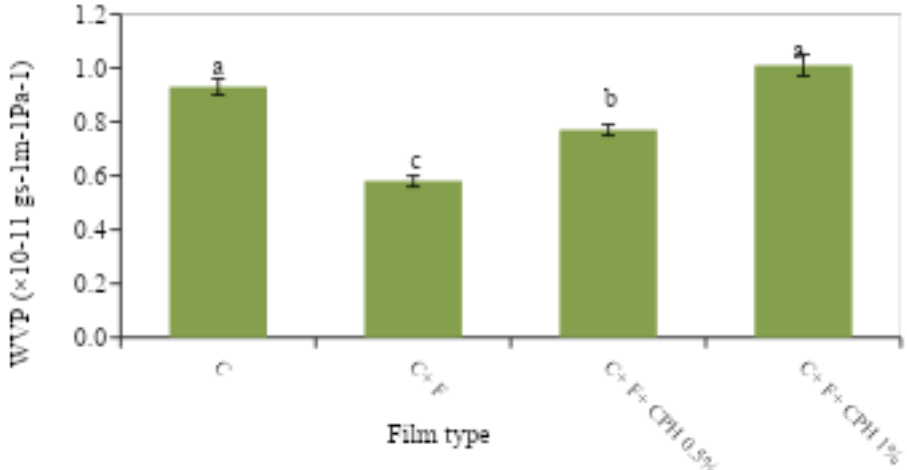

Fig 3. WVP of composite film along with crucian carp protein hydrolysates

3-6-Investigation of the mechanical properties of films

Tensile strength is one of the key parameters examined during the preparation of films, indicating their ability to resist tearing when exposed to external forces. According to the results (Figure 4), the addition of fucoidan to chitosan films improved their mechanical properties, which is due to the formation of hydrogen bonds between fucoidan and chitosan.[34], which leads to a change in the film structure and an increase in its tensile strength compared to films of chitosan alone. In general, the use of a crosslinking agent can improve mechanical properties such as swelling rate and tensile strength and lead to the formation of new chemical bonds and increased internal connections between polymer chains.[35]. These changes increase the strength and elasticity of the films, making them more suitable for various applications, including food packaging. Similar results were obtained byFurniture et al. (2023) andCaesar et al. (2007) observed in association with the addition of fucoidan to chitosan film.[10، 36]The addition of hydrolyzed protein to the films resulted in a decrease in the tensile strength of the film. (05/0P<).This decrease in tensile strength for chitosan-fucoidan films containing hydrolyzed protein indicates the brittleness of these films. In other words, these films are mechanically weaker and less deformable than films without protein. It is likely that these peptides are easily incorporated into the protein structure and form hydrogen bonds with the chains of the composite film. These interactions may be detrimental to the interchain bonding, leading to a decrease in the density of intermolecular interactions and an increase in the free space between the film chains. As a result, the tensile strength of chitosan-fucoidan films containing protein is reduced.[36].

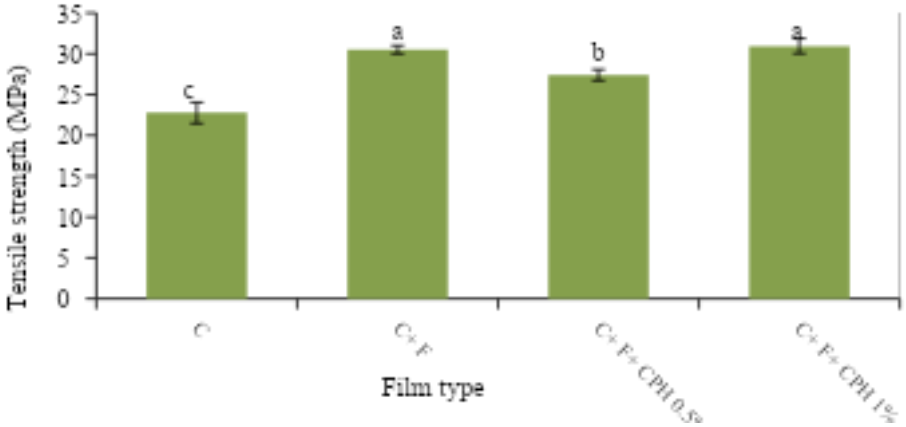

Fig 4. Tensile sterngth of composite film along with crucian carp protein hydrolysates

3-7-Investigation of the antioxidant properties of films

Based on the results, the addition of fucoidan to the chitosan film increased the antioxidant property of the film (Figure 5). This increase in antioxidant activity is attributed to the ability of fucoidan to donate hydrogen atoms, which is due to the presence of sulfate and hydroxyl groups in its structure. These groups can react with free radicals and also enhance the antioxidant stability of the film by forming hydrogen bonds and three-dimensional networks.[37].The results of the present study showed that adding hydrolyzed protein to the films improved their antioxidant properties.(05/0P<).Increasing the concentration of these proteins has a positive effect on the inhibition of DPPH free radicals (Figure 5). The antioxidant activity of hydrolyzed proteins depends on several reasons, such as their ability to eliminate free radicals, chelate metals, deoxygenate and donate hydrogen, as well as prevent the penetration of lipid oxidation initiators by forming a layer around oil droplets. According to the study of Pirveisi et al. (2023), nanocellulose film containing hydrolyzed pine seed protein has the ability to inhibit DPPH free radicals, which also increases with increasing protein concentration.[37].

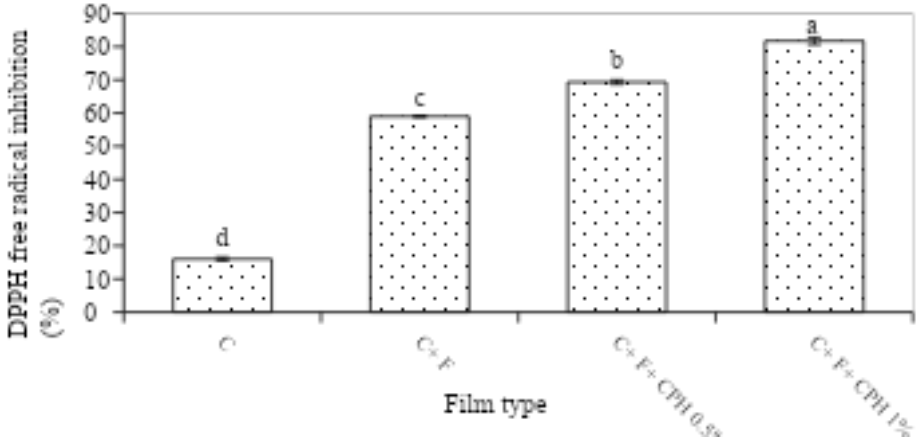

Fig 5. Antioxidant activity of composite film along with crucian carp protein hydrolysates

3-8-Investigating the antimicrobial properties of films

According to the results (Table 3), chitosan film had effective antimicrobial activity against both tested bacteria. There are two main theories to explain the mechanism of antimicrobial effect of chitosan. First, chitosan can chelate essential metals and elements through its polycationic property and remove them from the reach of bacteria. This action reduces the ability of bacteria to utilize these vital elements. Second, chitosan helps to destroy the cell wall of bacteria by forming bonds with anions of their cell wall. These two mechanisms give chitosan the ability to be used as a natural antimicrobial agent in the food and pharmaceutical industries. [39]. The antimicrobial activity of chitosan was increased by the addition of fucoidan ($P<0.05$). The antimicrobial activity of fucoidan is attributed to various compounds including amino acids, terpenoids, phlorotannins and phenolic compounds. Due to its chain structure, fucoidan can penetrate the bacterial wall or form a gel on their membrane, which helps to prevent food exchange and exert inhibitory effects on bacterial pathogens. Given the increasing resistance of bacteria to common antibiotics, the search for new antimicrobial compounds is of great importance, and fucoidan has been considered as a potential source of antimicrobial drugs [40, 41, 43]. The addition of hydrolyzed protein to the films led to an increase in their antimicrobial activity ($P<0.05$). In particular, the highest antimicrobial activity against both bacteria was observed in the composite film of chitosan, fucoidan, and 1% hydrolyzed protein. Antimicrobial peptides with membrane permeability function are able to penetrate into the bacterial membrane and thereby disrupt it. These changes cause an imbalance in cellular contents and disrupt the processes of DNA sequence replication, transcription, and translation by binding to specific intracellular targets [32 , 39 , 42].

Tabble 3: Antimicrobial activity of composite film along with crucian carp protein hydrolysates

Film / Microorganism	*Staphylococcus aureus* (mm)	*Escherichia coli*(mm)
C	6.65± 0.25^{d}	5.22± 0.33^{d}
C+ F	15.58± 0.45^{c}	13.58± 0.75^{c}
C+ F+ CPH 0.5%	18.35± 0.69^{b}	16.28± 0.95^{b}
C+ F+ CPH 1%	21.44± 1.20^{a}	18.18± 0.75^{a}

Data are given as mean± standard divition (n=3)
Different small letters in the same column indicate significant differences ($P<0.05$).

4- Conclusion

This study showed that adding hydrolyzed crucian carp protein to chitosan-fucoidan composite film can improve film properties. The results of mechanical

tests showed that increasing protein concentration reduced the tensile strength of the films. Also, the results of physical tests showed that protein increased the moisture content, water vapor permeability and turbidity of the films. The hydrolyzed protein also had high antioxidant and antimicrobial properties. Overall, this study shows that 1% hydrolyzed crucian carp protein can be used as a suitable additive in chitosan-fucoidan composite film.

5-Resources

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مقاله علمی_پژوهشی

تولید فیلم مرکب کیتوزان و فوکوئیدان استخراج شده از جلبک قهوه‌ای *Sargassum latifolium* به همراه پروتئین هیدرولیز شده ماهی کاراس (*Carassius carassius*)

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چکیده

هدف این مطالعه بررسی ویژگی‌های فیلم خوراکی مرکب کیتوزان و فوکوئیدان همراه با پروتئین‌های هیدرولیز شده ماهی کارارس می‌باشد. در این راستا، پروتئین هیدرولیز شده ماهی کاراس به وسیله آنزیم آلکالاز تولید و فوکوئیدان از جلبک قهوه‌ای *Sargassum latifolium* استخراج گردید. سپس چهار نوع فیلم خوراکی شامل کیتوزان و کیتوزان-فوکوئیدان با غلظت‌های مختلف پروتئین هیدرولیز شده (۰/۵، ۱ درصد) تهیه و ویژگی‌های آن‌ها مورد بررسی قرار گرفت. نتایج نشان داد که پروتئین هیدرولیز شده دارای میزان پروتئین و درجه هیدرولیز بالایی است. همچنین، بازده استخراج فوکوئیدان ۵/۸۹ درصد، کربوهیدرات کل ۵۹/۲ درصد، پروتئین ۹/۲۱ درصد، سولفات ۲۱/۷۸ درصد و اسید اورونیک ۸/۸۵ درصد بود. نتایج آزمون‌های مکانیکی نشان داد که افزایش غلظت پروتئین منجر به کاهش مقاومت کششی فیلم‌ها شد. به‌علاوه، آزمون‌های فیزیکی نشان داد که افزایش غلظت پروتئین باعث افزایش نفوذپذیری بخار آب و رطوبت فیلم‌ها گردید ($p<0/05$). پروتئین هیدرولیز شده ماهی کاراس دارای فعالیت قابل توجهی در مهار رادیکال‌های آزاد DPPH بود و افزایش غلظت آن تأثیر مثبتی بر این پارامتر داشت ($p<0/05$). همچنین، این فیلم‌ها خاصیت ضد میکروبی بالایی در برابر باکتری‌های پاتوژن داشتند و خاصیت ضد میکروبی آن‌ها علیه باکتری *استافیلوکوکوس اورئوس* بیشتر از *اشریشیاکلی* بود. به‌طور کلی، نتایج این مطالعه نشان می‌دهد که فیلم‌های خوراکی مرکب کیتوزان-فوکوئیدان با افزودن پروتئین‌های هیدرولیز شده می‌توانند به‌عنوان یک گزینه مناسب برای بهبود کیفیت و ماندگاری محصولات غذایی مورد استفاده قرار گیرند. این فیلم‌ها نه تنها خواص فیزیکی و مکانیکی بهتری دارند، بلکه فعالیت‌های آنتی‌اکسیدانی و ضد میکروبی آن‌ها نیز می‌تواند به افزایش ایمنی و کیفیت مواد غذایی کمک کند.