Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Study of the Effect of Hydrolyzed Protein from Turkmen Melon Seeds on Some Properties of Polyethylene/Nanoclay Nanocomposite Films

Gelareh Izadi Amoli [1], Peiman Ariaii[1*], Mahro Esmaeili[1], Roya Bagheri[1]

1-Department of Food Science and Technology, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

ARTICLE INFO

Article History:

Received:2024/9/16

Accepted:2024/11/10

Keywords:

Pathogenic bacteria,

Bioactive peptides,

Mechanical properties,

Edible films,

Enzymatic hydrolysis.

DOI: 10.22034/FSCT.22.165.118.

*Corresponding Author E-

p.aryaye@yahoo.com

ABSTRACT

Due to increasing concerns regarding the negative impacts of non-biodegradable packaging materials, the use of biodegradable packaging solutions is gaining significant attention. This study aims to evaluate the effects of hydrolyzed protein from Turkmen melon seeds utilizing the enzyme Protamax at concentrations of 0.5% and 1% (w/v) on the mechanical properties as well as the physicochemical, antioxidant, and antimicrobial characteristics of polyethylene/nanoclay-based nanocomposite films. The results demonstrate that the hydrolyzed protein has a high protein content and a notable degree of hydrolysis. Furthermore, this protein is rich in hydrophobic amino acids (35.26%) and aromatic amino acids (19.67%). The incorporation of nanoclay into polyethylene films resulted in reduced moisture content and water vapor permeability (WVP), along with increased thickness and opacity. In contrast, the addition of hydrolyzed protein led to increases in the thickness, moisture, and WVP of the films while decreasing opacity ($p < 0.05$). It was also observed that the tensile strength of the films decreased, while the elongation at break significantly increased with the addition of hydrolyzed protein. The hydrolyzed protein from melon seeds exhibited considerable DPPH free radical scavenging activity, with higher concentrations positively influencing this property ($p < 0.05$). Additionally, these films displayed stronger antimicrobial properties against pathogenic bacteria, notably showing greater efficacy against the gram-positive bacterium *Staphylococcus aureus* compared to the gram-negative bacterium *Escherichia coli*. The nanocomposite film containing 1% hydrolyzed protein demonstrated the highest antioxidant and antimicrobial activity ($p < 0.05$). Overall, the use of hydrolyzed protein from melon seeds in the preparation of nanocomposite films can lead to the development of suitable packaging materials for food applications. These films exhibit desirable physicochemical and mechanical properties, alongside effective antioxidant and antimicrobial characteristics.

1-Introduction

The simplest and most economical plastic made by polymerization of ethylene is polyethylene. Due to its low cost and functional properties, this material is widely used as film and food coating. Polyethylene has good barriers against water vapor, but it is not very effective against the penetration of oxygen and carbon dioxide [1]. Polymer nanocomposites are two-phase systems that include a polymer matrix and inorganic nanoparticles. Clay nanoparticles are one of the most studied nanoparticles. The reason for this attention is their cheapness, easy access and good performance and processability [2]. In a research, Hamzeh-kalkenari et al. (2021) stated that nanocomposite films containing nanoclay have more inhibitory properties than ordinary polyethylene films. Also, these researchers announced that when clay nanoparticles are added to nanocomposite films, it increases the strength of the film and decreases the permeability of the nanocomposite film [1].

Today, various types of antimicrobial compounds are used in the design of food packaging and coatings, and bioactive peptides are considered an important group of them. Bioactive peptides are known as parts of proteins that exist passively in the structure of primary proteins and contain 20 to 30 amino acids and their molecular weight is less than 6,000 daltons [3]. These peptides are produced through enzymatic, chemical or fermentation methods. In the meantime, the method of enzymatic hydrolysis of protein sources has attracted the attention of researchers due to its advantages such as favorable conditions, optimal production, less production of by-products and preservation of amino acids [4]. Hydrolysis of proteins involves breaking them down into smaller peptides and free amino acids. Enzymes used for protein hydrolysis may be of plant, animal and microbial origin [5]. Protamax enzyme is a protease produced by bacteria *Bacillus subtilis* It is widely used in food industry and biotechnology due to its ability to produce protein with high degree of hydrolysis in a short time and with less bitterness than other enzymes [6, 7].

Turkmen melon (*Cucumber melon* var. reticulata) belongs to the gourd family, which has high medicinal and nutritional properties. This fruit is rich in protein, fat, carbohydrates and mineral elements, especially iron, sodium and potassium [8]. The seeds of this product contain natural antioxidants and can be used as a source of nutrients and health-promoting compounds [9]. Currently, melon seeds are usually only used in limited nut and animal feed applications, and most of them are discarded as waste. Therefore, applying additional processes on these wastes and producing different products with added value can be an effective solution to reduce waste and increase economic efficiency [8, 10].

According to the studies done, there has been no research on the effect of hydrolyzed protein of Turkmen melon seeds in polyethylene/nanoclay nanocomposite film. Therefore, the aim of this research is to analyze the physical, mechanical, antioxidant and antimicrobial properties of this type of nanocomposite with the addition of hydrolyzed Turkmen melon seed protein.

2- Materials and methods

2-1- Raw materials

In this research, the low density polyethylene polymer of Bandar Imam Petrochemical was used. Silver nanoparticles with a size of 35 nm were purchased from Neutrino China. Folin Ciocaltive reagent and free radicals 2 and 2 diphenyl 1-picrylhydrazyl (DPPH) was obtained from Sigma Aldridge, USA. Other chemicals used were of laboratory purity and produced by German Merck and American Sigma Aldridge companies.

2-2- Defatting from seeds

200 grams of Turkmen melon seeds were dried, ground and then mixed with hexane solvent at a ratio of 1:10 (volume-weight) and stirred for 4 hours by a shaker at a speed of 440 rpm. Next, the remaining solvent was separated from it by oven under vacuum at 30 degrees Celsius for 2 hours. Then the resulting powder was passed through a 40 mesh sieve [11].

2-3 Hydrolyzed protein production

Checking the protein content of melon seed samples was done by Keldahl method. In this method, the samples were first subjected to digestion and then the amount of total nitrogen in the samples was determined using titration. Finally, using the conversion factor of 6.25, the total amount of protein in the aqueous phase of the samples was calculated [12]. For hydrolysis of melon seed protein, protein with a concentration of 4% (weight/volume) was prepared in phosphate buffer 7 (optimal pH) for Protamax enzyme. Hydrolysis was performed at an optimal temperature of 50°C, with an enzyme concentration of 1% and a time range of 10 minutes to 1 hour in a Shakerdar incubator (V-8480, South Korea) at 200 rpm. To deactivate the activity of the enzymes, the protein solution was placed in a hot water bath with a temperature of 80°C for 15 minutes. Then, the solution was centrifuged at 10,000 rpm for 20 minutes. To produce hydrolyzed protein powder, the resulting supernatant was dried in a freeze dryer (5509 FDB Operon, South Korea) at -20 °C, 40 mbar pressure, and 48 hours. The hydrolyzed protein powder was stored at -20°C until use [13].

2-5 degrees of hydrolysis

The degree of hydrolysis was calculated based on the amount of α amino acid in the amount of protein in the sample [4].

2-6 amino acid composition

In order to synthesize the amino acid of the hydrolyzed protein powder, the protein sample was first completely hydrolyzed for 24 hours at 110°C using 6 M hydrochloric acid. Then the available amino acids were derivatized by adding phenyl isothiocyanate (PITC). The amount of total amino acids was measured using a Smart line HPLC device (made in Germany) equipped with a C18 column and a fluorescent detector (RF-530). [5].

2-7 preparation of nanocomposite film

At first, the powder of nanoclay particles (density 1.98 g/ml and average particle size 35 nm) and polyethylene granule (with low density along with concentrations of 0.5 and 1% hydrolyzed protein of melon seeds) were physically mixed. Then the prepared mixtures were passed through a double helix aligned extruder (with a diameter of 19 mm and a length to diameter ratio of 40) to mix in a molten state. In the molten state, the temperature of the extruder was set from 180 to 200 degrees Celsius and the spiral speed was set to 100 to 160 revolutions per minute. In the next step, the film production machine of Brabander Alman was used in this machine. The output of the machine was set to 250 cm A thickness of 20 microns was prepared [1].

2-8-Measuring the physical properties of films

1-8-2-Measuring the thickness of films

A micrometer with an accuracy of 0.01 mm was used to measure the thickness. The thickness was measured at 5 points of the film and its average value was reported [14].

2-8-2 Measuring the moisture content of films

Film samples with specific weight were placed in glass plates that had already reached a constant weight and were weighed (W1). Then it was dried in the oven at 105 degrees Celsius for 24 hours. The sample with the plate was removed after this period and after cooling in the desiccator, it was weighed again (W2). The moisture content of the films was calculated based on wet weight from Equation 1 [15].

Relationship 1

$$100\times W1/ (W2-W1) = \text{moisture percentage}$$

3-8-2-Permeability against water vapor

In order to measure the permeability of films to water vapor (ASTM E 96-02), first 10 milliliters of distilled water was poured into the permeability measurement cells, and then the glass cells whose surface was sealed by film and grease were placed in a desiccator containing silica gel. Water at a temperature of 25 degrees Celsius creates 100% humidity. The difference in humidity on both sides of the film at a temperature of 25 degrees Celsius creates a heater pressure difference equal to 2.337.103 pascals. Cell weight changes were measured over time using a digital scale with an accuracy of 0.0001 grams. The water vapor transfer rate in terms of (gram)-meter-second was equivalent to the slope of the resulting lines divided by the cell surface and was obtained from equation 2 [19]. The area of the cells was 0.00287 square meters. From multiplying the water vapor transmission rate (WVTR) by the film thickness (L) and dividing it by the pressure difference on the two sides of the film (AP), the water vapor permeability (WVP) $(10)^{-11}$ $gs^{-1}m^{-1}Pa$) was obtained [15].

Relationship 2

Cell surface (meters) / slope of the line (g/s) = water vapor transfer rate (g^{1-} $seconds^{2-}$ meters)

5-8-2-turbidity

To determine the turbidity, the films prepared as pieces with dimensions of cm^2 1 x 4 was cut. Then these parts were placed in the cell of the spectrophotometer and their absorbance was read at a wavelength of 600 nm. Equation 3 was used to determine the turbidity. [15].

Relationship 3

100x absorbance = turbidity

2-9-Mechanical properties of films

The mechanical tests of the films are based on the modified method of ASTM D0882-02. The films are cut into pieces of 761 cm and conditioned under the conditions of relative humidity of 50% and temperature of 25 degrees Celsius. Their thickness is determined at 5 measurement points and their average thickness. The mechanical properties of the film (extensibility (percentage), tensile strength (MPa)) were measured using Instran. In Instran, the distance between two jaws is 50 mm, the movement speed of the upper jaw is 50 mm/min and the lower jaw is fixed [15].

2-10- Antioxidant activity of films

The antioxidant activity of the film was measured using the DPPH free radical scavenging method [16]. For this purpose, first, 25 mg of the film was gently mixed in 3 ml of distilled water. Then, 8.2 ml of this solution was added to test tubes containing 0.2 ml of 1 mM DPPH solution in methanol and kept at room temperature for 30 minutes. The optical absorption of the samples and the control sample was measured at a wavelength of 517 nm using a spectrophotometer. The decrease in light absorption compared to the control indicated the ability of the compounds in the film to inhibit the DPPH free radical. Finally, the percentage of inhibitory activity of DPPH free radicals was calculated using equation 4.

Relationship 4:

100] × (absorption rate of control / absorption rate of sample - absorption rate of control) - 1 = DPPH free radical scavenging percentage

2-11- Determination of antimicrobial activity of films

Bacterial cultures *Staphylococcus aureus* (PTCC 1189) and *Escherichia coli* (PTCC 2019) was prepared from the microbial collection of Tehran University. Using a sterile loop, an aliquot of each bacterium was removed from sterile ampoules and added to 10 mL of BHI Broth culture medium. The inoculated culture medium was placed in a greenhouse at 37°C for 24 hours. After incubation, bacteria were cultured on nutrient agar plates using a sterile loop. The cultured plates were kept in a greenhouse at 37°C for 24 hours. 3 to 5 isolated and homogenized colonies were transferred to tubes containing 5 ml of physiological serum using a sterile swab. The turbidity (optical absorption) of the bacterial suspensions was measured at a wavelength of 625 nm using a spectrophotometer. Suspensions to a concentration equal to half McFarland, which is equivalent to approx 10^8 Colony per ml, diluted. Using a sterile swab, the bacterial suspensions were uniformly spread on the surface of the culture media. Round discs of film with a diameter of 6 mm were cut using a round knife. The film discs were placed at suitable intervals on the plates impregnated with bacteria and the plates were incubated for 24 hours at 37°C. After incubation, the diameter of the transparent halos around the film discs was measured and reported in millimeters. This method was used to investigate the antimicrobial activity of edible films using the diffusion method in agar [17].

12-2- Statistical evaluation

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

3- Results and discussion

3-1- degree of hydrolysis

The results of the present study showed (Table 1) that with the increase in the time of the hydrolysis process, the degree of hydrolysis, which indicates the breaking of peptide bonds, increases ($P<0.05$). In other words, the longer the hydrolysis time, the more proteins are broken down into smaller peptides. However, with increasing time and degree of hydrolysis, the intensity and rate of separation of soluble proteins from non-soluble components decreases. This shows that the more the peptide bonds are broken and the proteins are converted into smaller peptides, the more soluble they become and they leave the insoluble state [5, 18]. By increasing the hydrolysis time from 90 to 120 minutes, probably inhibition of substrate release led to saturation of the reaction rate, so that no significant difference was observed in the degree of hydrolysis ($P>0.05$). In these conditions, with the passage of time and the reduction of substrate amounts, the degree of hydrolysis is affected [19]. Islam et al. (2022) reported similar results on the degree of hydrolyzation of hydrolyzed soybean protein by Protamax enzyme. They found that with increasing storage time, the degree of hydrolysis increases but reaches a constant value after some time [19].

2-3-The amount of protein

The primary protein content of melon seeds is 0.54±was 26.86%, after degreasing with hexane, the amount of protein was 1.20±It has increased by 41.41%. Enzymatic hydrolysis using Protamax enzyme has caused a significant increase in the amount of protein between 76.12-92.71% (Table 1). This improvement in the amount of protein by enzymatic hydrolysis, due to the reduction of fat, moisture and the removal of impurities¬during the process of protein extraction with centrifugation [3, 19]. Therefore, enzymatic hydrolysis can be an effective method to improve the quality and increase the amount of protein of melon seeds, and based on the results, melon seeds are considered a valuable source of protein, which can be increased by using the hydrolysis process. Compared to other studies conducted on melon seeds, the amount of protein in this study was more or equivalent [8، 10].

Table 1: Degree of hydrolysis and protein content of turkmen melon seed protein hydrolysates at different hydrolysis time

Hydrolysis time (min)	Degree of hydrolysis (%)	Protein content (%)
20	12.41±0.51[and]	76.12±1.49[and]
40	28.95±0.95[d]	85.55±0.86[d]
60	35.31±0.40[c]	88.24±0.52[c]
80	43.41±0.80[b]	90.73±0.62[b]
100	47.03±0.50[a]	92.94±0.34[a]
120	47.01±1.52[a]	92.71±0.38[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

Amino acids, as compounds with antioxidant properties, play an important role in maintaining the quality and shelf life of food. By neutralizing free radicals and preventing the oxidation of fats and other sensitive molecules in food, these compounds prevent spoilage and adverse changes in the taste, smell and color of food products [20]. In the present study (Table 2), high amounts of essential and non-essential amino acids were detected, especially tryptophan with 10.22% (the most essential amino acid) and glutamic acid with 20.45% (the most non-essential amino acid) were reported as higher concentrations. These findings are in line with the results of the study of Mallek-Ayadi et al. (2019) on the profile of amino acids in melon seeds. *Cucumber melon* is In that study, tryptophan and glutamic acid were identified as the dominant essential and non-essential amino acids [21]. The study showed that the concentration of all essential amino acids, except phenylalanine, was beyond the recommended values (FAO/WHO, 1990) for the needs of adults [22]. This rich pattern of essential amino acids indicates the high nutritional value of melon seeds, which can play an effective role in meeting the basic needs of amino acids in adult diets. The sum of hydrophobic and aromatic amino acids in hydrolyzed proteins was 35.26 and 19.67%, respectively. The presence of these amino acids at optimal levels indicates their high nutritional value. Also, these amino acids are responsible for the functional and biological properties of proteins, including antioxidant, anti-inflammatory, anti-cancer activity and reducing sugar and blood pressure [23].

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

Amino acid(g 100 g^{-1})	Protamex	FAO/ WHO, 1990
Histidine [a]	1.67	1.9
Isoleucine [a]	4.98	2.8
Leucine[a]	8.55	6.6
Lysine[a]	3.11	5.8
Methionine[a]	1.58	
Phenyl alanine[a]	5.87	6.3
Threonine[a]	4.01	1.1
Valine[a]	2.99	3.5
Tryptophan	10.22	
Arginine	3.15	
Aspartic acid	8.44	
Glycine	3.44	
Proline	3.56	
Serine	3.11	
Alanine	4.15	
Cysteine	5.11	
Glutamic acid	20.45	

Tyrosine	3.58
Asparagine	1.05
Total amino acid	95.01
LET[b]	35.26
AAA[c]	19.67

a Essential amino acids
b Total hydrophobic amino acids (alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, proline, methionine and cysteine)
c Total amount of aromatic amino acids (phenylalanine, histidine, tryptophan and tyrosine)

3-4- Investigating the physical properties of films

The humidity index represents the total occupied volume of the network microstructure of the film by water molecules, while the solubility index is related to the degree of hydrophilicity of the films [24]. According to the results (Chart 1), the highest moisture values were observed in polyethylene treatment. The addition of nanoclay decreased the moisture content of the film ($P<0.05$). The decrease in humidity in nanocomposite films in comparison with the control film is probably due to the reduction of capacity and empty spaces in the polymer substrate and actually related to the phenomenon of compression of the structural network of the film due to the presence of nanoclay [2]. But the addition of hydrolyzed protein caused a significant increase in moisture values. is ($P>0.05$). This increase in moisture can be due to the hydrophilic and buffering properties of hydrolyzed protein. Hydrolyzed proteins are able to hold more water in their structure and can also moderate humidity fluctuations. As a result, adding these compounds to the product has increased the final moisture content [3].

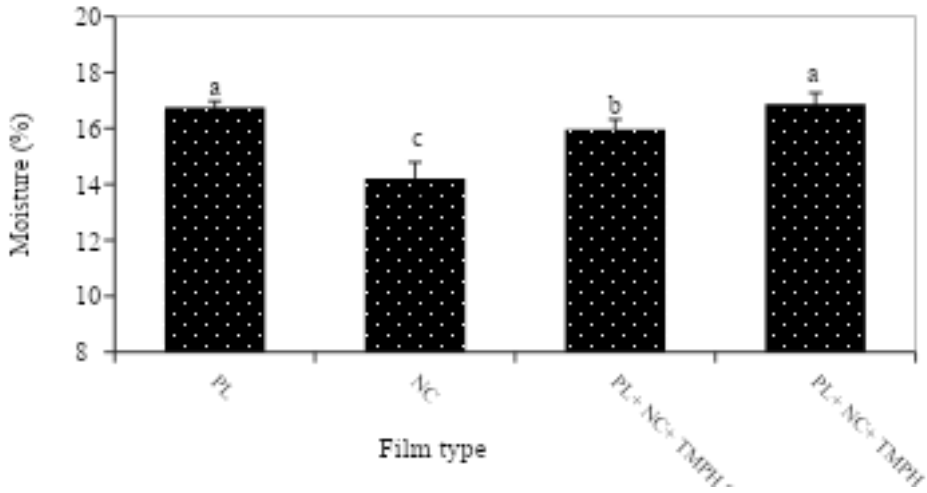

Fig 1. Moisture content of nano-composite film along with hydrolyzed melon seed protein

The thickness of edible films depends on the concentration of their constituents, the amount of initial solution of the film per unit area, and the speed of pouring this solution on the surface. This feature has a great impact on important factors in the evaluation of films, such as permeability to water vapor and their mechanical behavior. In other words, the thickness of edible films is affected by the concentration of ingredients, the amount of initial solution of the film per unit area and the speed of pouring this solution on the surface, and these factors play a very important role in determining the permeability to water vapor and the mechanical properties of the films [25]. According to the results (Chart 2), the lowest thickness values were in polyethylene film. By adding nanoclay and hydrolyzed protein to the film, the thickness increased so that the highest thickness was observed in the film of polyethylene + nanoclay

+ hydrolyzed protein 1% ($P < 0.05$). The increase in the thickness of edible films is due to the addition of nanoclay and hydrolyzed protein. The thickness of the film depends on its nature and composition. This effect of the nature and composition of the film on the thickness can be seen in the results of other studies. For example, de Oliveira et al. (2019) and Ghasemi et al. (2021) reported similar results on films containing hydrolyzed protein. In general, the feature¬Film properties such as thickness are significantly affected by the composition and nature of its constituents [26, 27].

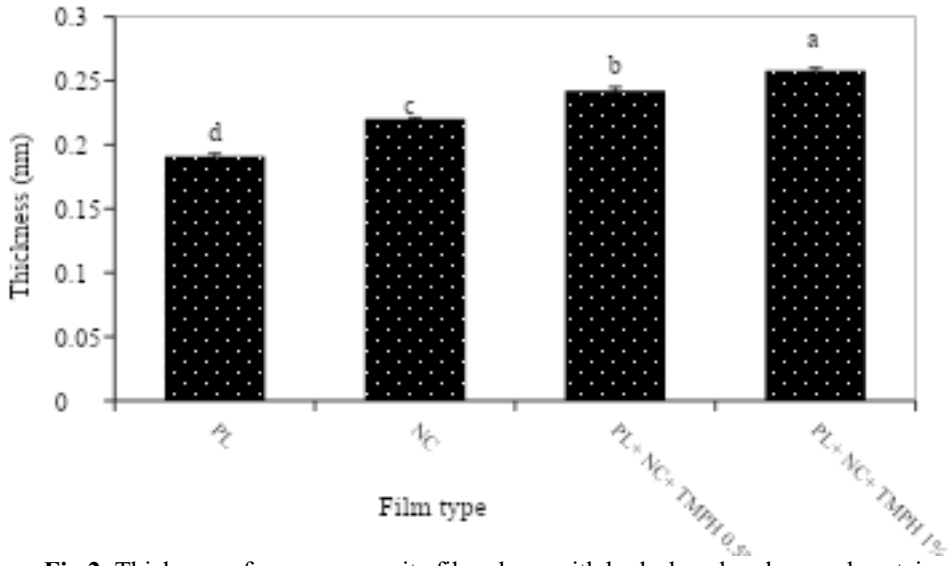

Fig 2. Thickness of nano-composite film along with hydrolyzed melon seed protein

The results indicate (Chart 3) that the addition of nanoclay in the polyethylene film caused a significant decrease in the amount of WVP ($P<0.05$). In the present research, the reduction of WVP in edible films can be due to the creation of a tortuous space by clay nanoparticles, followed by the closing of the fine paths in the film structure. In other words, the presence of clay nanoparticles in the composition of the film creates a more complex structure in the film, which prevents the easy passage of water vapor molecules through it. This more complex structure and the closing of the micro-pathways in the film ultimately lead to a decrease in the WVP of the film [28]. This conclusion is in accordance with the study conducted by Ojagh et al. (2018), which investigated the role of clay nanoparticles in reducing the permeability to water vapor of double-layer edible agar/fish gelatin films containing titanium dioxide nanoparticles. By adding hydrolyzed protein to the film, the permeability increased [29]. This increase in WVP is due to the plasticizing effect of low molecular weight hydrolyzed protein. The presence of this protein has led to the increase of hydrophilic groups in the film structure, and subsequently, the presence of more water molecules in the film structure has increased the permeability to water vapor. Also, increasing the thickness of the hydrolyzed protein layers has also affected the WVP values. Similar results have been observed in the studies of de Oliveira et al. (2019) on alginate film containing hydrolyzed protein of cottonseed, Ghasemi et al. (2021) on carboxymethyl cellulose film containing hydrolyzed protein of silver carp fish skeleton [26, 27].

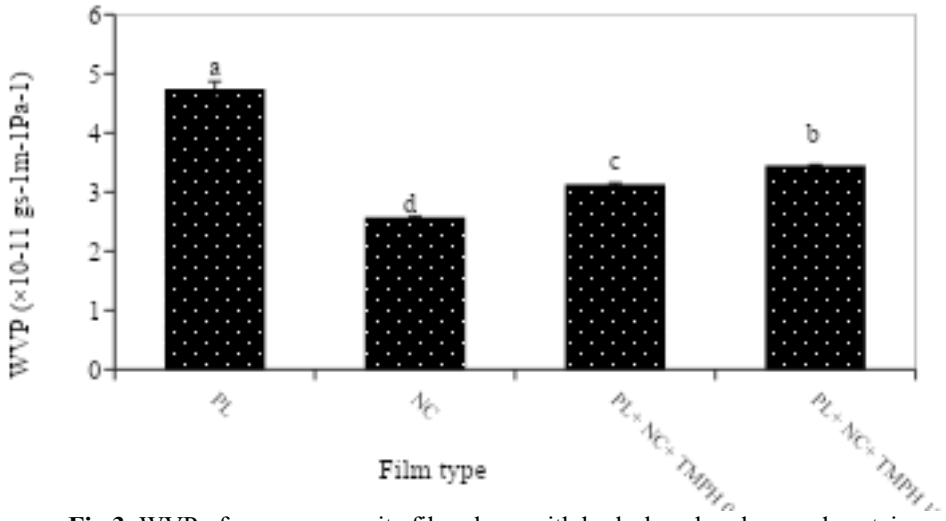

Fig 3. WVP of nano-composite film along with hydrolyzed melon seed protein

Optical properties of films, such as color, transparency, and light transmission, are among the important properties that affect film opacity and darkness. These optical properties can affect the appearance, acceptance, marketing and suitability of films for various applications. According to the results, adding nanoclay to polyethylene significantly reduced the film's opacity (Chart 4) ($P<0.05$). These results are consistent with the results of Rhim and Wang (2014) regarding the addition of clay nanoparticles to carginan film. Also, the addition of hydrolyzed protein also increased the turbidity of the film [30]. Adding hydrolyzed protein to edible films can increase the thickness of the film and thus reduce its transparency and increase its turbidity [27]. This phenomenon occurs due to more scattering of light in the film due to the accumulation of hydrolyzed protein [26]. Although reducing transparency can lead to reduced consumer demand, reducing the speed of visible light transmission can be beneficial for food packaging. Because the presence of light can cause changes in color and taste, loss of nutrients and finally oxidative spoilage of food. Therefore, the balance between optical properties, including transparency and opacity, should be considered in the design of food packaging films.

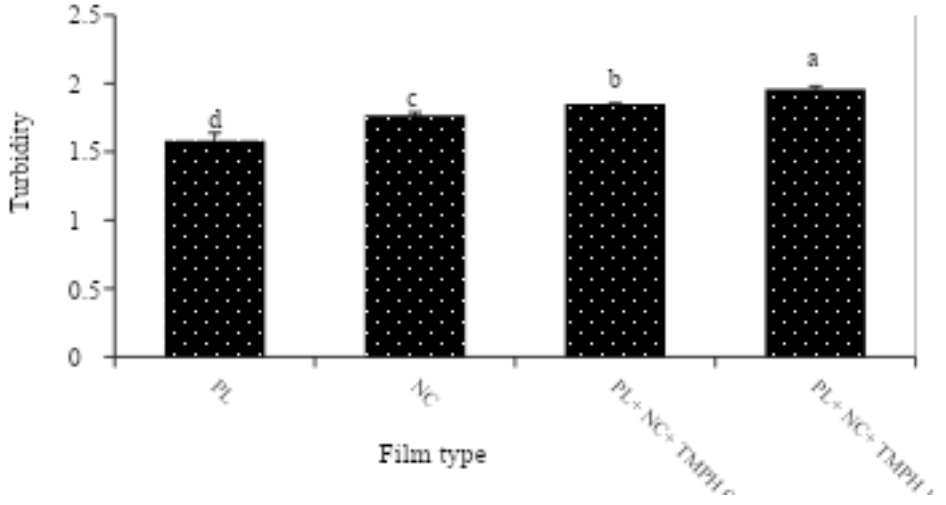

Fig 4. Turbidity of nano-composite film along with hydrolyzed melon seed protein

3-5- Checking the mechanical properties of the films

Tensile strength of films is defined as the maximum stress required to tear the film during the tensile test. Tensile strength is one of the important mechanical criteria of the film, which is obtained by dividing the results of the tensile strength with the results of the length of the film at the moment of tearing to the length of the film due to the application of tensile pressure, which is expressed in MegaPascal units. These important features are important in the applications of food packaging and other industrial uses of films [29]. With the addition of nanoclay, the tensile strength of the films increased (Chart 5) and the maximum tension before the breaking point (Chart 6) decreased ($P<0.05$). It may be due to the homogeneous distribution of clay nanoparticles in polyethylene, so that nanoclay in these concentrations as a filler strengthens the properties of the polymer [29]. But the addition of hydrolyzate protein decreased the tensile strength and increased the maximum tensile strength before the breaking point ($P<0.05$). The decrease in tensile strength for polyethylene-nanoclay films containing hydrolyzed protein indicates the brittleness of these films. This means that these films are mechanically weaker and less deformable than the protein-free film. This behavior can be attributed to the interaction between polyethylene chains and small peptides resulting from protein hydrolysis. Probably, these peptides are easily included in the protein network and establish hydrogen bonds with the nanofilm chains. This is detrimental to the interaction between the chains and leads to a decrease in the density of intermolecular interactions and an increase in the free volume between the chains of the nanofilm. As a result, the tensile strength of polyethylene-nanoclay films containing protein decreases [31].

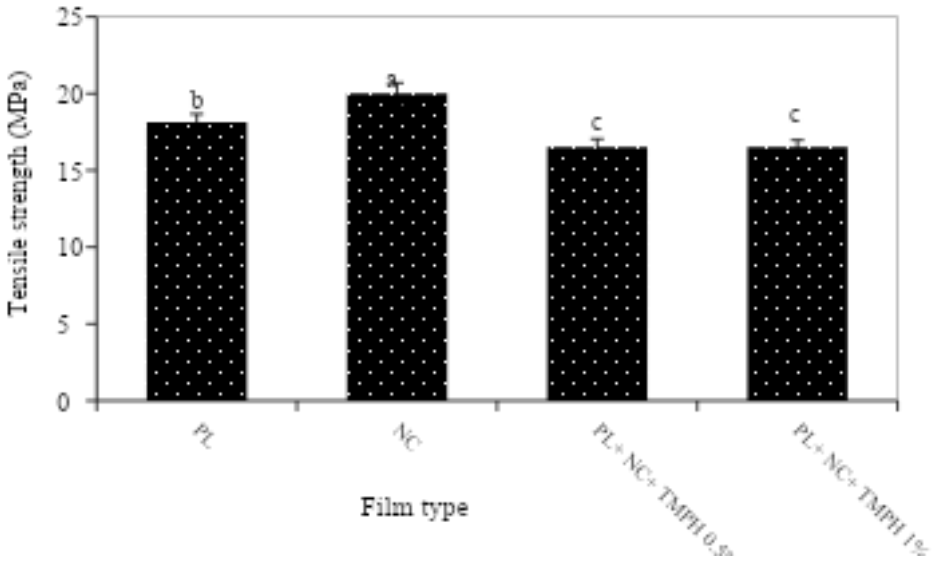

Fig 5. Tensile sterngth of nano-composite film along with hydrolyzed melon seed protein

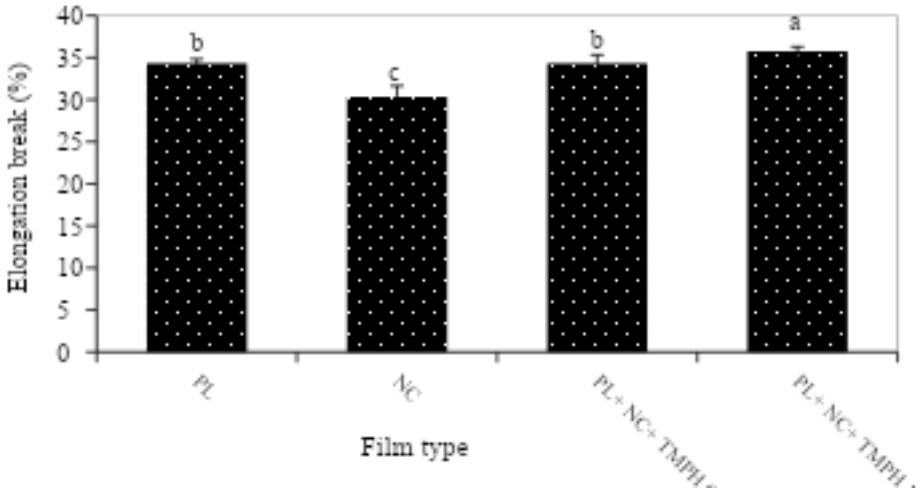

Fig 6. Elongation breaks of nano-composite film along with hydrolyzed melon seed protein

6-3- Investigating the antioxidant properties of films

Measurement of antioxidant activity is usually done through various techniques that directly or indirectly measure the rate and extent of free radical formation or destruction. More precisely, the various tests used to measure antioxidant activity are based on the fact that oxidation is largely inhibited by controlling the initiating or progressive free radicals in the spontaneous oxidation process. Therefore, these experiments focus on monitoring the ability of additives to scavenge radicals or prevent radical formation, rather than monitoring the oxidation process itself [32]. The results of the present study showed (Chart 7) that adding hydrolyzed protein to the films improves their antioxidant properties. Increasing the concentration of these proteins has a positive effect on inhibiting DPPH free radicals ($P<0.05$). The antioxidant activity of hydrolyzed proteins is due to several reasons, such as their ability to remove free radicals, act as metal chelators, oxygen quenchers and hydrogen donors, as well as the possibility of preventing the penetration of fat oxidation initiators through the formation of layers around oil droplets. Other studies have also shown that hydrolyzed proteins and plant bioactive peptides have antioxidant properties in laboratory conditions [13, 33, 34]. Also, based on the study of Pirveisi et al. (2023), nano cellulose film containing hydrolyzed pine seed protein has the ability to inhibit the DPPH free radical, which increases with increasing protein concentration [35].

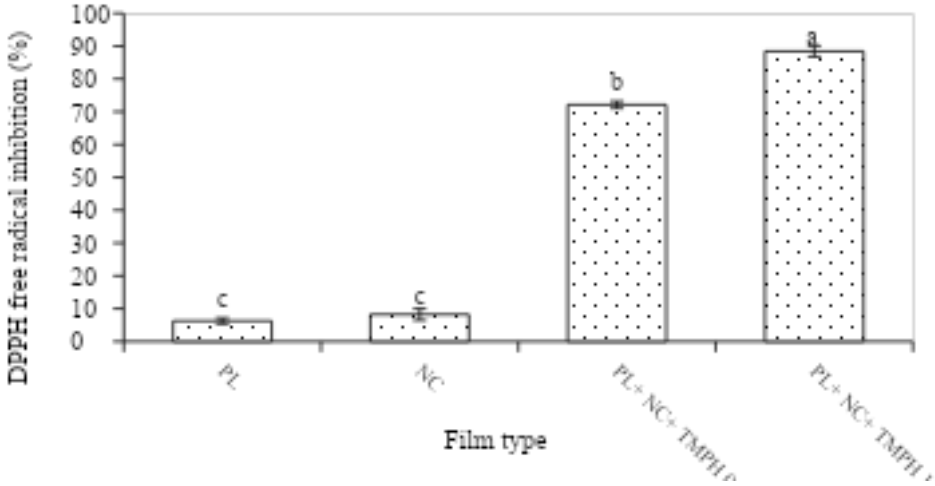

Fig 7. Antioxidant activity of nano-composite film along with hydrolyzed melon seed protein

7-3- Investigating the antimicrobial properties of films

Based on the results, polyethylene film and polyethylene-nanors had no significant antimicrobial properties (Chart 8). But with the addition of hydrolyzed protein, the antimicrobial property of the film increased significantly. So

that the highest level of antimicrobial property of the films against both bacteria was observed in polyethylene film + nanoclay + 1% hydrolyzed protein ($P < 0.05$). The antimicrobial peptides in this formula can disrupt the cell contents and disrupt important processes such as replication, transcription and translation by penetrating the bacterial cell membrane. This mechanism reduces the growth and finally the death of bacteria [26, 36, 37]. It also has antimicrobial properties against gram-positive bacteria *Staphylococcus aureus* higher than gram-negative bacteria *Ashirshyakali* was Various reports have shown that Gram-positive bacteria are more sensitive to antibacterial compounds than Gram-negative bacteria. This high sensitivity of gram-positive bacteria is due to the absence of lipopolysaccharide cell wall, which in gram-negative bacteria may prevent the penetration of active compounds into the cytoplasmic membrane. The resistance of gram-negative bacteria to antibacterial substances is related to the hydrophilic surface of the outer membrane of bacteria, which is rich in lipopolysaccharide molecules and creates a barrier against the penetration of various antibiotic molecules. Also, the enzymes of the periplasmic space in these bacteria are able to break the molecules introduced from outside. Gram-positive bacteria do not have such an outer membrane in the cell wall structure [37].

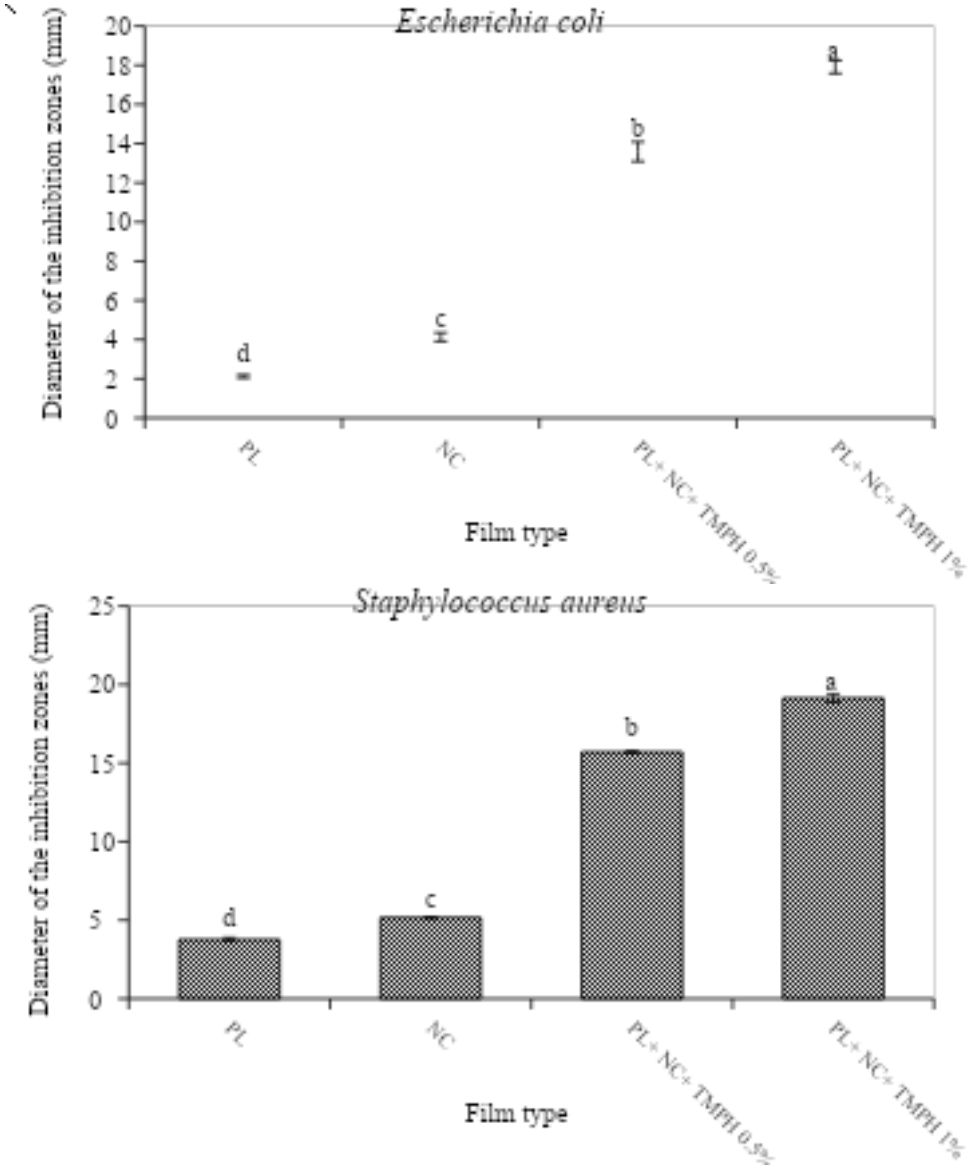

Fig 8: Influence of hydrolyzed melon seed protein on the antimicrobial activity of nano-composite film

4- Conclusion

This research showed that the addition of hydrolyzed protein of Turkmen melon seeds to nanocomposite films based on polyethylene and nanoclay has a positive effect on the mechanical, physical, antioxidant and antimicrobial properties of these films. By increasing the concentration of hydrolyzed protein up to 1%, the films showed better performance in terms of antioxidant and antimicrobial properties. This innovation can lead to the production of suitable films for food packaging, which have favorable characteristics and are compatible with the environment. In summary, the hydrolyzed protein of melon seed can be used as an effective additive in polyethylene nanoclay films.

5-Resources

[1] Hamzeh-Kalkenari, S., Bodaghi, H. & Ghasimi Hagh, Z. (2021). Improving postharvest quality of button mushroom (Agaricus bisporus) using polyethylene-clay nanocomposite packing and Echinophora cinerea essential oil coating. Iranian Food Science and Technology Research Journal, 17(2): 315-328.

[2] Abdollahi, M., Rezaei, M. &Farzi, G. (2012). Improvement of active chitosan film properties with rosemary essential oil for food packaging. International Journal of Food Science and Technology, 47: 847–853.

[3] Nemati, M., Shahosseini, S. R., & Ariaii, P. (2024). Review of fish protein hydrolysates: production methods, antioxidant and antimicrobial activity and nanoencapsulation. Food Science and Biotechnology, 33, 1789–1803.

[4] Ovissipour, M., Rasco, B., Shiroodi, S. G., Modanlow, M., Gholami, S., & Nemati, M. (2013). Antioxidant activity of protein hydrolysates from whole anchovy sprat (Clupeonella engrauliformis) prepared using endogenous enzymes and commercial proteases. Journal of the Science of Food and Agriculture, 93, 1718-1726.

[5] Shahosseini, S. R., Javadian, S. R., & Safari, R. (2022). Effects of molecular weights-assisted enzymatic hydrolysis on antioxidant and anticancer activities of Liza abu muscle protein hydrolysates. International Journal for Peptide Research & Therapeutics, 28, 72.

[6] Nemati, M., Javadian, S. R., Ovissipour, M., & Keshavarz, M. (2012). A study on the properties of alosa (Alosa caspia) by-products protein hydrolysates using commercial enzymes. World Applied Sciences Journal, 18(7), 950-956.

[7] Nemati, M., Javadian, S. R., & Keshavarz, M. (2019). Production of protein hydrolysates from Caspian shad (Alosa caspia) by-products using Alcalase enzyme. Journal of Marine Biology, 11(43), 87-95.

[8] Alvand, M., sadeghi mahoonak, A., ghorbani, M., shahiri tabarestani, H., & kaveh, S. (2022). Comparison of the Antioxidant Properties of Hydrolyzed Turkmen Melon Seed Protein by Pancreatin and Alcalase. Food Engineering Research, 21(2): 75-90.

[9] Zeb, A. (2016). Phenolic profile and antioxidant activity of Melon (Cucumis melo L.) seeds from Pakistan. Foods. 5: 67–74.

[10] Shahidi, F., Kochaki, A., & Baghaii, H. (2006). Investigation of some chemical compounds and physical properties of watermelon, squash, cantaloupe and melon seeds native to Iran and determination of chemical properties of their oil. Journal of Agricultral Science and Technology. 20(5): 421-411.

[11] Feyzi, S., Varidi, M., Zareb, F. & Varidi, M.J. (2015). Extraction Optimization of Fenugreek Seed Protein. Science of Food and Agriculture. 15: 3165–3176.

[12] AOAC. (2005). Official Method of Analgsis (17th ed). Washington, DC: Association of Official Analytical chemists.

[13] Mirzapour, Z., Ariaii, P., Safari, R., et al. (2022). Evaluation the effect hydrolyzed canola meal protein with composite coating on physicochemical and sensory properties of chicken nugget. International Journal of Peptide Research and Therapeutics, 28, 97.

[14] Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chemistry, 122(1), 161-166.

[15] ASTM. (1996). Standard test methods for tensile properties of thin plastic sheeting, D882-91. Annual book of ASTM. Philadelphia, PA: American Society for Testing and Material.

[16] Siripatrawan, U., & Harte, B. R. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloids, 24(8), 770-775.

[17] Emiroğlu, Z. K., Yemiş, G. P., Coşkun, B. K., & Candoğan, K. (2010). Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. Meat Science, 86(2), 283-288.

[18] Liaset, B., Nortvedt, R., Lied, E., & Espe, M. (2002). Studies on the nitrogen recovery in enzymatic hydrolysis of Atlantic salmon (Salmo salar, L.) frames by Protamex™ protease. Process Biochemistry, 37(11), 1263-1269.

[19] Islam M, Huang Y, Islam S, Fan B, Tong L, & Wang F. (2022) Influence of the Degree of Hydrolysis on Functional Properties and Antioxidant Activity of Enzymatic Soybean Protein Hydrolysates. Molecules. 19;27(18):6110.

[20] López-García G, Dublan-García O, Arizmendi-Cotero D, & Gómez Oliván LM. (2022). Antioxidant and Antimicrobial Peptides Derived from Food Proteins. Molecules. 16;27(4):1343.

[21] Mallek-Ayadi S, Bahloul N, & Kechaou N.(2019). Phytochemical profile, nutraceutical potential and functional properties of Cucumis melo L. seeds. J Sci Food Agric. 99:1294-1301.

[22] Food and Agriculture Organization of the United Nations;World Health Organization; United Nations University. Protein and Amino Acid Requirements in Human Nutrition: Report of a Joint FAO/WHO/UNU Expert Consultation; FAO: Rome, Italyd; WHO: Geneva, Switzerland, 2007.

[23] Gao , Y. Wang , Y. Yan and Z. & Li , J. (2021). Ultrasonic-alkali method for synergistic breakdown of excess sludge for protein extraction, Cleaner Prod., 295, 126288.

[24] Jiang, Y. F., Li, Y. X., Chai, Z., & Leng, X. J. (2010). Study of the physical properties of whey protein isolate and gelatin composite films. J. Agric. Food Chem., 58, 5100-5108.

[25] Hosseini, A., & Moradinezhad, F. (2018). Effect of short-term high CO2 treatment on quality and shelf life of button mushroom (Agaricus bisporus) at refrigerated storage. Postharvest Biology a Technology, 1, 37-48.

[26] de Oliveira Filho, J.M., Rodrigues, J.M., Valadares, A.C.F., de Almeida, F.D.L., & Dyszy, F.H. (2019). Active food packaging: Alginate films with cottonseed protein hydrolysates. Food Hydrocolloids, 92, 267-275.

[27] Ghasemi, Z., Alizadeh Khaled-Abad, M., Almasi, H., & Nikoo, M. (2022). Carboxymethyl cellulose based bioactive edible films with Lactobacillus casei and fish protein hydrolysates. Iranian Food Science and Technology Research Journal, 17(6), 85-102.

[28] Rhim, J.W., Wang, L.F. & Hong, S.I. (2013). Preparation and characterization of agar/silver nanoparticles composite films with antimicrobial activity. Food Hydrocolloids, 33(16): 327-335.

[29] Ojagh, S. M., Vejdan Taleshmikaeil, A., Abdollahi, M. (2018) Effect of nanoclay addition on the properties of agar/fish gelatin bilayer film containing TiO2 nanoparticles. Iranian Food Science and Technology Research Journal, 14(1): 27-38.

[30] Rhim, J. W., and Ng, P. K. 2014. Natural biopolymer-based nanocomposite films for packaging applications. Critical reviews in food science and nutrition, 47(4): 411-433.

[31] Kchaou, H., Jridi, M., Benbettaieb, N., Debeaufort, F., & Nasri, M. (2020). Bioactive films based on cuttlefish (Sepia officinalis) skin gelatin incorporated with cuttlefish protein hydrolysates: Physicochemical characterization and antioxidant properties. Food Packaging and Shelf Life, 24, 100477.

[32] Benbettaïeb N, Debeaufort F, & Karbowiak T. (2019). Bioactive edible films for food applications: mechanisms of antimicrobial and antioxidant activity. Crit Rev Food Sci Nutr. 59(21):3431-3455.

[33] Ghanbarinia, S. H., Ariaii, P., Safari, R., & Najafian, L. (2022). The effect of hydrolyzed sesame meal protein on the quality and shelf life of hamburgers during refrigerated storage. Animal Science Journal, 93(1), e13729.

[34] Mirsadeghi Darabi, D., Ariaii, P., Safari, R., & Ahmadi, M. (2022). Effect of clover sprouts protein hydrolysates as an egg substitute on physicochemical and sensory properties of mayonnaise. Food Science & Nutrition, 10, 253–263.

[35] Pirveisi, N., Ariaii, P., Esmaeili, M., Lotfi, L., Kaboosi, H., & Afshar Sedigh, R. (2023). Investigating active packaging based on cellulose nanofibers oxidized by TEMPO method containing hydrolyzed protein obtained from pine tree fruit on the quality of pacific white shrimp (Litopenaeus vannamei) during the storage period. Food Measurement, 17, 3323-3337.

[36] Tkaczewska, J. (2020). Peptides and protein hydrolysates as food preservatives and bioactive components of edible films and coatings - A review. Trends in Food Science & Technology, 106, 298-311.

[37] Ghafari, E., Ariaii, P., & Bagheri, R. (2024). Investigating the effect of nanochitosan-Iranian tragacanth gum composite film along with Eryngium campestre essential oil on the shelf life of goat meat. Food Measurement, 18, 1543-1558.

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

مطالعه تاثیر پروتئین هیدرولیز شده دانه خربزه ترکمنی بر برخی ویژگی‌های فیلم نانوکامپوزیت پلی‌اتیلن/ نانورس

گلاره ایزدی آملی[۱] ، پیمان آریایی*[۱]،مهرو اسماعیلی[۱]، رویا باقری[۱]

۱-گروه علوم و صنایع غذایی، واحد آیت ا... آملی، دانشگاه آزاد اسلامی،آمل، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۶/۲۶

تاریخ پذیرش: ۱۴۰۳/۸/۲۰

کلمات کلیدی:

باکتری پاتوژن،

پپتیدهای زیست فعال،

خواص مکانیکی،

فیلم خوراکی،

هیدرولیز آنزیمی

DOI: 10.22034/FSCT.22.165.118.

* مسئول مکاتبات:

p.aryaye@yahoo.com

چکیده

امروزه، به دلیل نگرانی‌های فزاینده درباره‌ی تأثیرات منفی مواد بسته‌بندی غیرقابل تجزیه، استفاده از مواد بسته‌بندی تجزیه‌پذیر به طور فزاینده‌ای مورد توجه قرار گرفته است. . هدف از این پژوهش به بررسی اثر پروتئین هیدرولیز شده دانه خربزه ترکمنی با استفاده از آنزیم پروتامکس (۰/۵ و ۱ درصد وزنی/ حجمی٪) بر خواص مکانیکی، فیزیکی، آنتی‌اکسیدانی و ضد میکروبی فیلم نانوکامپوزیت بر پایه پلی‌اتیلن و نانورس می باشد. نتایج نشان می‌دهد که پروتئین هیدرولیز شده دارای میزان پروتئین بالا و درجه هیدرولیز قابل توجهی است. این پروتئین غنی از اسیدهای آمینه هیدروفوب (۳۵/۲۶٪) و اسیدهای آمینه آروماتیک (۱۹/۶۷٪) می‌باشد. افزودن نانو رس به فیلم پلی‌اتیلن منجر به کاهش رطوبت و WVP و افزایش ضخامت و کدورت گردید. با این حال، افزودن پروتئین هیدرولیز شده باعث افزایش ضخامت، رطوبت و WVP فیلم‌ها و کاهش کدورت شد (P<۰/۰۵). همچنین، مشاهده گردید با افزودن پروتئین هیدرولیز شده، مقاومت کششی فیلم‌ها کاهش یافته و ازدیاد طول نمونه‌ها به‌طور معناداری افزایش یافته است (P<۰/۰۵). پروتئین هیدرولیز شده دانه خربزه فعالیت قابل توجهی در مهار رادیکال‌های آزاد DPPH نشان داد، و افزایش غلظت آن تأثیر مثبتی بر این خصوصیت داشت (P<۰/۰۵). این فیلم‌ها همچنین خاصیت ضد میکروبی قوی‌در برابر باکتری‌های پاتوژن داشتند، اثر آن‌ها بر باکتری گرم مثبت *استافیلوکوکوس اورئوس* بیشتر از باکتری گرم منفی *اشریشیا کلی* بود. فیلم نانوکامپوزیت حاوی ۱٪ پروتئین هیدرولیز شده بیشترین فعالیت آنتی‌اکسیدانی و ضد میکروبی را نشان داد (P<۰/۰۵). به‌طور کلی، استفاده از پروتئین هیدرولیز شده دانه خربزه در تهیه فیلم نانوکامپوزیت می‌تواند به تولید فیلم‌های مناسب برای بسته‌بندی مواد غذایی منجر شود. این فیلم‌ها ویژگی‌های فیزیکی و مکانیکی مطلوبی دارند و دارای خواص آنتی‌اکسیدانی و ضد میکروبی مطلوبی نیز می‌باشند.