



Original research

## Physical and mechanical features investigation of protein-based biodegradable films obtained from trout fish waste

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### ABSTRACT

Biological packaging material based on obtained proteins from fish waste, are biopolymers that have the capability of biodegradable film formation. Thus, purpose of this research is to study and investigate some of films' physical features made from trout fish Myofibril protein. The film forming solution containing 1.5%, 2% and 2.5% (w/v) Myofibril protein isolate of 100ml solution and glycerol as plasticizer in three levels 25%, 50%, 75% (w/w) per dry material have been used. In order to solve existing protein in solution its pH must be reach to 3 by 1molar HCl. Films have been produced by casting method for 48 hours in 25°C. Then film's mechanical features, water vapor penetrability, films solubility in water, transparency and color were investigated. Effect of protein concentration was significantly obvious on films mechanical features, thickness, water vapor penetrability and color. Obtained results from statistical analysis data by SPSS software showed that films with 2% (w/v) dry material along with 50% (w/w) glycerol demonstrated the best mechanical features.

**Keywords:** Trout fish waste, Edible film, Mechanical properties, Water vapor permeability, Scanning electron microscope

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## 1. Introduction

Material used for packaging, are biological contaminant. Plastic with oil resource like polyolephins, polyester sand polyamides due to availability in large amount and reasonable price and sufficient functional characters are used widely as packaging material, however these compounds are not biodegradable and cause environmental pollution (Tharanathan 2003).

Films and edible coatings are in the form of edible thin layers which put on food surface or inside of its constituents and are determined as one of basic strategies to control the physiological, microbial and physiochemical changes in food. Their main structure is based on natural polymers having specific characters and the main constituents of films and edible coatings include: lipids, proteins and polysaccharides (Krochta, 2002).

Recently a range of studies accomplished on and about edible films and coatings based on polysaccharides, lipids, protein or a combination of them with following advantages: degradability in nature, selective penetrability and possibility of vapor transferring control, oxygen and Co<sub>2</sub> transmission control, using edible films and coatings as their potential ability to provide a combination of moisture, oxygen, taste, smell, color and edible oil inhibitory features along with their quality and shelf life of food increase.

In many cases, the most important features of film with edible coats are fighting against moisture transfer. Because specific levels of water activity should be preserved in a lot of foods, destructive enzymes and chemical reactions are greatly under the effect of water activity or amount of moisture. Moisture transfer speed between food and surrounding atmosphere decreases by complete covering of food using edible film or coating. In addition to vapor transfer, transfer of some gas like oxygen and CO<sub>2</sub> affects the food shelf life (Siracusa et al. 2008).

Generally, mechanical and inhibitory features of protein-based films are widely from polysaccharides films. Because unlike polysaccharides which are homopolymer, proteins have particular structure (based on 20 types various Amino acids) that causes a vast ranges of potential functional characters of these films, especially increase of molecular bonds. They are able to establish plenty of different bond types and various energy that provide solubility conditions and pH in different area of protein bonds as a function of temperature. According to the obtained result from Kinsella (1976), Chou and Morr (1979), and Cheftel et al. (1985) studies, experimental parameters like protein concentration, pH, temperature, time, ionic strength, and different additive percent, causes the change of protein-protein and protein-water reactive power that leads to change of functional features. Effects of these

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parameters on Myofibril gel forming features and film formation have been studied by [Lavelle and Foegeding \(1993\)](#) and [Wu and Bates \(1972\)](#), respectively. Furthermore, in order to improve their features, raw protein material can be changed chemically ([Osawa & Walsh, 1993](#)).

Proteins with high molecular weight are generally insoluble or poorly soluble in water, so there are molecules which are very interested in water resistant film forming. Among these proteins, myofibril proteins are able to form unusual films. Myofibril

proteins make up more than 50% of muscle weight. They include contractible proteins (actin and myosin) and regulating muscles contraction proteins (tropomyosin, troponin, actin, ...) which can be identified through their unusual features. Formation of protein films requires complete solution of protein by regulating pH of film forming solution using appropriate soluble ([Tahergorabi et al., 2012](#)). Purpose of this research is to investigate the mechanical, optical, WVP and solubility features of films made of trout fish myofibril protein.

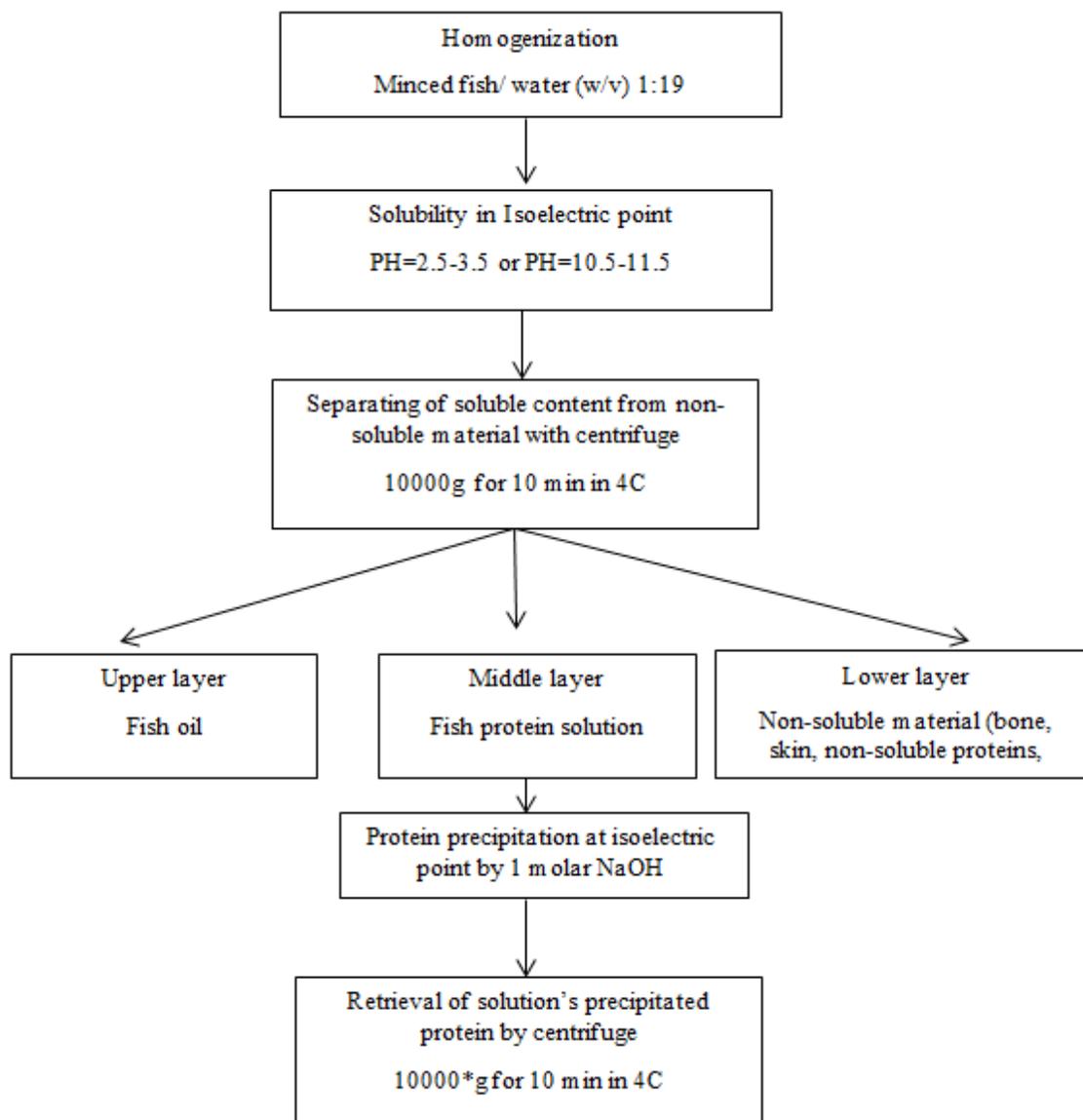


Fig. 1. Retrieval of fish isolated protein using solubility/ precipitation at isoelectric point.

## 2. Material and Methods

### 2.1. Material

Glycerol with 98% purity from Acros company (England) used in all formulation as a plasticizer. In order to solve the myofibril proteins, sodium hydroxide (NaOH) 97% purity and chloridric acid (HCL) 37% purity bought from Merk Company (Germany). To produce relative humidity 53% Magnesium sulfate and calcium chloride (Calcium chloride dehydrate) to regulate the activity of water (aw) in 0.75 provided from Merk company (Germany) and sodium azid to inhibit the microorganism growth have been used.

### 2.2. Fish sample

The farm fresh trout with average weight of 380-450 g approximate length of 18-29 cm bought from a local market. Fish have been put in ice in ratio of 1:2 (fish/ice) and after 30 min at the maximum was send to the laboratory. Then sample preparation stages have been done immediately.

### 2.3. Sample preparation and protein recycle

A trout (including skin, scale and bone) used to recycle the muscle protein isolate by solubility/precipitation process described by [Nolsøe and Undeland \(2009\)](#) in [Fig. 1](#). protein solution existing in water and effected by mixed pH ([Nolsøe & Undeland, 2009](#)). Firstly, fish minced by meat grinder (ModelMT-1000 Pars TOSHIBA industrial co/Iran) with 4.95 mm mesh three times. Sample mixed with cold ionized stilled water in ratio of 1:9 and mixed by a mixer (Sunny. model: SFP-820/Iran) 820 W for 1 min. During the process, sample temperature modified by being put in ice 4°C ([Tahergorabi et al., 2012](#)). Prepared sample homogenized by ultratorax homogenizer (IKA-T25 digital) 10000 round per minute for 1 min. Secondly, myofibril and skeletal protein with modifying mixed pH solved in pH = 2.5-3.5. Under these condition all the sarcoplasmic proteins solved and unsolved parts are only parts of connective tissue proteins, membranous protein, and probably denatured sarcoplasmic and myofibril protein. In the third stage soluble part separated from insoluble part by centrifuge 10000 g round in 4°C for 10min. Then in the fourth stage by arranging pH in isoelectric point myofibril protein precipitate in pH = 5.5 ± 0.5 using NaOH 1 molar and in the last stage protein sediment separated by centrifuge 10000g round in 4°C for 10 min ([Nolsøe & Undeland, 2009](#)).

### 2.4. Film solution preparation

To prepare film from three concentrations 1.5%, 2%, 2.5% (w/v) of dry material along with plasticizer in concentration 25% (w/w of protein), 50% and 75% were used. The solution of film preparation developed by Paschoalick and colleagues ([Paschoalick et al., 2003](#)). So the forming solution compound includes: 1.5%, 2%, 2.5% (w/v) protein dry material (gram per 100ml film forming solution), glycerol as plasticizer in three levels 2.5%, 50%, 75% (w/v) (gram glycerol to gram protein) have been used. The pH solution regulated using chloridric acid (1molar) reached the point 3. Then the film solution was poured in a Teflon plate and in an entire balanced level to obtain films with homogeneous surface

having identical thickness in all parts, put in an 30°C incubator fan equipped (to move the air) for 48 hours.

### 2.5. Thickness

Thickness of unconditional films using a digital Micrometer (Mitutoyo, LIC.No.689037-Japan) with precision 0/0001mm in 5 different points (4 in circumference and 1 in center) of films measured in random and were averaged.

### 2.6. Moisture content

Pieces of totally homogenous films after arriving to equilibrium moisture weighed by a digital scale with precision 0.0001 and placed inside a capsule that reached a constant weight in advance. Then to reach a constant temperature is put in 105°C for 24 hours. In this test glycerol evaporation from film considered negligible. Then according to the samples weight reduction in compare to initial sample, film moisture percent identified. This test repeated 5 times and the obtained data mean reported as the film moisture.

### 2.7. Solubility in water

Solubility in water of edible films determined by Gontard and colleagues method ([Gontard, 1994](#)). After determination of film moisture value, content of existing solid material were identifiable. According to it, following the film reaches the equilibrium moisture and drying in 105°C oven for 24h, were heated to approach a constant weight and measured after drying. Then film pieces were put in 50 cc distilled water and to prevent microorganism growth 0.02 (w/v) azid sodium was added. Then the sample container was put in 25°C for 24h under slow stirring condition, the mixture of film and water filtered on a filter paper which previously reached the constant weight and measured. Filter paper along with samples placed in a constant temperature of 105°C to reach a constant weight.

### 2.8. Water vapor permeability

Penetrability measurement test accomplished using the modified ASTM method No E96. To perform the present test waterless calcium chloride poured inside the glass cell. Then, the cell's surface covered by melted paraffin. In this case 0% relative moisture exists in cells. Cells were placed in desiccator containing saturated NaCl. Saturated NaCl produced 75% moisture in 25°C. Differences in moisture on both sides of film recorded by a digital scale with 0/0001 accuracy and in all samples by drawing the graph of weight changes to time, graph drawn in the function time form. Slope of each drawn line figured by regression line ( $R = 0/999$ ) and rate of vapor transfer obtained by dividing the drawn slope line ( $\text{gs}^{-1}$ ) into film's surface ( $\text{m}^2$ ). WVP obtained by multiplying the film thickness and dividing to pressure differences between cell and desiccator's relative moisture.

### 2.9. Mechanical properties

Film mechanical tests accomplished using a Testometer equipment (Rochdale, Lncashire, England, M350-10ct, Testometric Co, Ltd). Films were cut rectangular size of 10 mm\*100 mm, according to standard method and due to low

thickness of samples and smallness of equipment jaw, there was no need to use dumbbell-shaped samples. Using standard method of ASTM (2001) the space between the equipment's jaws fixed 50%. Samples placed in 25°C and 50% relative moisture by Magnesium Nitrate saturated solution used for sample's conditioning. Tensile strength (TS) factors and elongation at break (EAB%) obtained from Stress- Strain graphs. Repeated 5 times for each samples.

### 2.10. color

In order to identify the film color a colorimeter Minolta model (Minolta, CR 300 Series, Minolta camera Co., Ltd, Osaka, Japan) used to measure L, b and a parameters. Prior to the film color measurement equipment fixed using an standard white plate as a plates with samples on it and the color standard parameters identified as L=84.71, a=+1.26, b=-3.58). Samples placed on standard white plate and the above parameters have been measured. Parameters that is showed by the equipment are: Light (0=black and 100=white) and a colorful parameters (+60=redness to -60=greenness) and b (+60=yellowness to -60= blueness). Three points selected in random for each film.

### 2.11. Transparency

Transparency measured using a Luxometer (Testo 540 pocket sized, UK). To do the present test initially the equipment bubble put under a light source with minimum modulation and the rate of crossing light noted according to the obtained lux and from two achieved numbers, the samples' transparency reported in percent. At least 5 repetitions considered for test of each film.

### 2.12. Study of morphology by scanning electron microscope (SEM)

Electron microscope images of film surface and cross section provided in order to investigate the effect of glycerol addition as plasticizer on produced film structure. To study the film's morphology made of fish myofibril protein along with plasticizer

the scanning electron microscope (Philips XL 30, Netherlands) having image analysis system with acceleration voltage of 15 kV have been used. Prior to sample imaging, films were cut in size of 5 mm × 1 mm by surgical blade, installed on a bronze plate and covered by a layer of gold in order to conduct the samples, and imaging done by enlarging 500 for surface and 800 for cross section of film.

### 2.13. Statistical analysis

Statistical calculation determined according to factorial test in a complete in random format by using variance analysis (ANOVA) in 5% probability level. Comparison of data mean using SPSS software version 17 based on Dunken test accomplished.

## 3. Results

### 3.1. Thickness

Existing dry material content in film surface unit, fitting the developed film's apparent thickness directly is from fish myofibril protein. It seems that order of chains which forms the protein matrix is a kind that establishes a constant density, even in cases that drying of film forming solution takes a longer time than usual.

As the films protein contents increases, due to polar groups that exist in protein's surface, it is capable to absorb the surrounding water. So through increase of protein dry material from 1.5% (w/v) to 2.5% (w/v) thickness increases significantly ( $p > 0.05$ ) (Table 1).

Effect of thickness changes on edible film mechanical and physical features is considerable. Obtained results from various data show that by increase of protein dry material content from 1.5% (w/v) to 2.5% (w/v) films thickness also increases from estimated values 0.052 mm, 0.059 mm and 0.069 mm, respectively, for 1.5%, 2% and 2.5% (w/v) ( $p < 0.05$ ).

Along with thickness increase, required power for tearing of films has also demonstrated a significant increase (Table 1).

Table 1. Trout myofibril protein films solubility, thickness and transparency in various dry material concentrations.

Protein concentration (w/v)	Glycerol concentration (w/w)	thickness(mm)	Solubility (%)	Transparency (%)
1.5	25	0.041±0.015 <sup>ef</sup>	11.3±0.46 <sup>f</sup>	96.57±0.09 <sup>ab</sup>
1.5	50	0.049±0.008 <sup>def</sup>	14.81±0.39 <sup>e</sup>	97.08±0.18 <sup>ab</sup>
1.5	75	0.059±0.006 <sup>bcd</sup>	19.88±0.44 <sup>c</sup>	97.44±0.22 <sup>a</sup>
2	25	0.046±0.012 <sup>cde</sup>	14.18±0.37 <sup>e</sup>	94.79±0.15 <sup>de</sup>
2	50	0.056±0.003 <sup>f</sup>	16.36±0.36 <sup>d</sup>	95.07±0.16 <sup>d</sup>
2	75	0.061±0.009 <sup>abc</sup>	19.92±0.64 <sup>c</sup>	96.16±0.27 <sup>bc</sup>
2.5	25	0.066±0.016 <sup>ab</sup>	19.49±0.47 <sup>c</sup>	93.98±0.12 <sup>e</sup>
2.5	50	0.069±0.007 <sup>abc</sup>	22.09±0.22 <sup>b</sup>	94.67±0.19 <sup>de</sup>
2.5	75	0.073±0.007 <sup>a</sup>	29.07±0.75 <sup>a</sup>	95.42±0.16 <sup>cd</sup>

Table 2. Color parameter including L, a and b of trout myofibril protein films in dry material and glycerol various concentrations.

Protein concentration (w/v)	Glycerol concentration (w/w)	b	a	L
1.5	25	-2.66±0.12 <sup>bcd</sup>	0.76±0.09 <sup>ab</sup>	70.27±2.18 <sup>a</sup>
1.5	50	-2.77±0.43 <sup>cd</sup>	0.8±0.16 <sup>ab</sup>	70.99±7.43 <sup>a</sup>
1.5	75	-2.82±0.61 <sup>d</sup>	0.83±0.12 <sup>a</sup>	71.94±4.22 <sup>a</sup>
2	25	-1.92±0.19 <sup>ab</sup>	0.62±0.06 <sup>abc</sup>	70.82±5.15 <sup>a</sup>
2	50	-1.99±0.34 <sup>ab</sup>	0.67±0.05 <sup>abc</sup>	72.41±3.84 <sup>a</sup>
2	75	-2.06±0.65 <sup>abc</sup>	0.69±0.09 <sup>abc</sup>	73.29±5.81 <sup>a</sup>
2.5	25	-1.41±0.13 <sup>a</sup>	0.50±0.01 <sup>c</sup>	73.81±4.14 <sup>a</sup>
2.5	50	-1.46±0.40 <sup>a</sup>	0.52±0.14 <sup>c</sup>	74.70±2.58 <sup>a</sup>
2.5	75	-1.52±0.57 <sup>a</sup>	0.57±0.04 <sup>bc</sup>	75.25±6.12 <sup>a</sup>

### 3.2. Transparency

Considering the application of edible films, transparency is proposed as one of the fundamental factors of edible film acceptance (Fang, 2006). The obtained films from myofibril protein are transparent enough to be used in packaging system. However, transparency measurement and its comparison with color index shows that fish myofibril protein films in low concentration of dry material 1.5% (w/v) are given high transparency (83.8) that decreases (36.2%) through the increase of dry material concentration 2.5% (w/v) and the light and transparent color of films tend towards yellowness and the increase of b parameter (Table 2) confirms this apparent change.

### 3.3. Solubility in water

Totally, adding of plasticizer to polymer material causes the change of three-dimensional molecule structure, decrease of molecular gravity and increase of free volume and molecule chains mobility (Ekrami & Emam-Djomeh, 2014).

Solubility in water increase of films, containing plasticizer, is the reflection of plasticizer solubility in water. The protein network solubility in water is not under the significant influence of plasticizer concentration. In other words, adding plasticizer to the myofibril protein-based films causes great reduction in film strength and tensile and also significant increase of transformation and WVP features. Difference in film solubility depends on their thickness.

Regardless the type and concentration of plasticizer, myofibril protein won't spread in water after 24 h and remains untouched in water. Increase in content of utilized plasticizer (regardless of its type), increase the content of soluble dry material in water and causes the linear increase in soluble dry material contents. In fact, it is the effect of hydrophilic plasticizer contents change that causes the increase of protein film solubility in water.

### 3.4. Mechanical properties

Through increase of dry material from 1.5% (w/v) to 2% (w/v) in per films surface unit, the samples tensile strength value increases from  $2.86 \times 10^{-11}$  to  $3.84 \times 10^{-11}$  (Fig. 2 and 3). Biological packaging based on fish myofibril protein, have the similar mechanical features to other biological-based packaging. Mechanical features of biological packaging based on trout myofibril protein are very close to physical features of polysaccharide films like tensile and breaking point for propyl

Methyl cellulose-based films and tensile strength for hydroxyl propyl methyl cellulose-based films (CUQ et al., 1995).

On the other hand myofibril protein relatively contains enormous content of glutamine and asparagine Amino acid (10 mol/100 mol amino acid). Replacement of double hydrogen bonds (85 kJ/mol) between two amid groups, with single hydrogen bonds (25 kJ/mol) between an amid group and a plasticizer molecule can also be another reason for molecular reaction density decrease in these films.

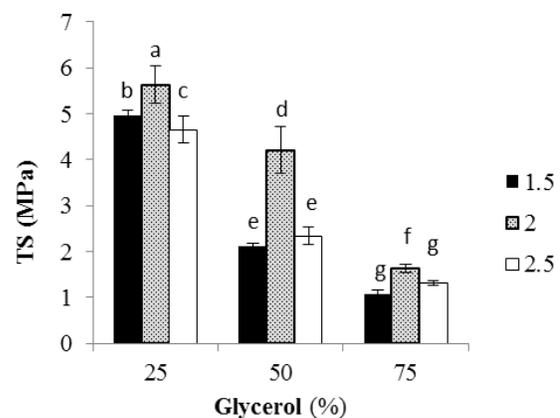


Fig. 2. Effect of various dry material and glycerol on tensile strength (TS) of trout myofibril protein films.

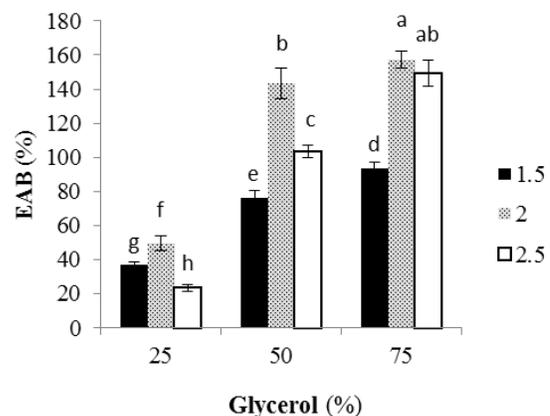


Fig. 3. Effect of various dry material and glycerol on elongation at break (%EAB) of trout myofibril protein films.

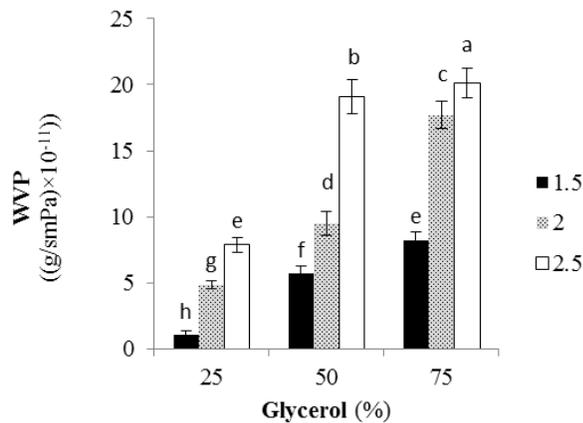


Fig. 4. Effect of various dry material and glycerol on water vapor permeability (WVP) on trout myofibril proteins.

### 3.5. Water vapor permeability (WVP)

Because of high hydrophilic groups in polymer chains, protein and carbohydrate edible films have low inhibition of water vapor, in proteins as non-polar Amino acid (Hydrophobic) is high in compare to polar Amino acid in their structure, inhibition will be more. As it is expected, through plasticizer concentration increase, enormous increase observes in WVP. Placement and connection of plasticizer inside matrix polymer networks causes change in polymer cohesive network construction so that causes the free space between polymer chains, thus polymer matrix has low density and as a result it will be more penetrable. Considering the obtained images from the films' cross section it is realized that in high dry material concentration, due to lack of adhesion in protein matrix, existing cracks, the WVP increases. Consider the Fig. 4 data, in proportion to dry material, data statistical analysis, shows WVP significant increase ( $p < 0.05$ ). Penetrability resulted from plasticizer concentration increase is due to plasticizer molecule hydrophilic feature.

In fish myofibril protein-based films a positive cohesion exists between thickness and WVP, though this relationship does not exist in ideal polymer-based films, because for ideal homogenous polymer, PHIC law requires the change of vapor leakage under a vapor pressure gradient inversely by the film's thickness (Hauser, 1948).

So, because of dry material content increase from 1.5% (w/v) to 2.5% (w/v) which accompanies the film thickness increase and also due to increase of hydrophilic groups increase and lack of appropriate solubility and film's homogeneity, WVP increases significantly ( $p < 0.05$ ).

### 3.6. Color

Regardless of the type and concentration of plasticizer, produced films from fish myofibril protein, are transparent and colorless (Table 2).

Glycerol concentration increase causes the reduction of film's color that is probably as the result of glycerol elution which is an independent and colorless material of protein concentration (do A Sobral et al., 2005).

Considering that solving protein in acidic condition happens with  $pH = 3$ , it is observed that acidic condition, stimulate the formation of yellow pigments, particularly those which are produced during Maillard reactions (Chinabark et al., 2007).

As the protein concentration increases more, it causes the increase of b parameter and decrease of a parameter.

### 3.7. Study of morphology by scanning electron microscope

Study of morphological features in polymers is an important factor to understand its behavior and features (Frinaut, 2006). Taken images of SEM from film's surface (50% (w/w) glycerol) give the beneficial data from the film's morphology, surface and cross section. Images obtained from fish myofibril protein-based films (Fig. 5 and 6) demonstrates a homogenized structure in which gathering of myofibril proteins forms a dense and consistent network which is also confirmed in other researches (Limpan, 2010).

Though, presence of a dense matrix is one of common features of protein films, increase of dry material content, additional protein that are not able to be solved in film forming solution are obvious in the form of insoluble ellipse-like clusters in cross section images (Fig. 7). Through increase of the film's dry material from 1.5% (w/v) to 2.5% (w/v) solubility decreases and this lack of solubility causes the decrease of film's adhesion and increase of WVP (Fig. 4) that behavior is observed in similar films which are produced from myofibril proteins of other fish (Sobral, 2007).

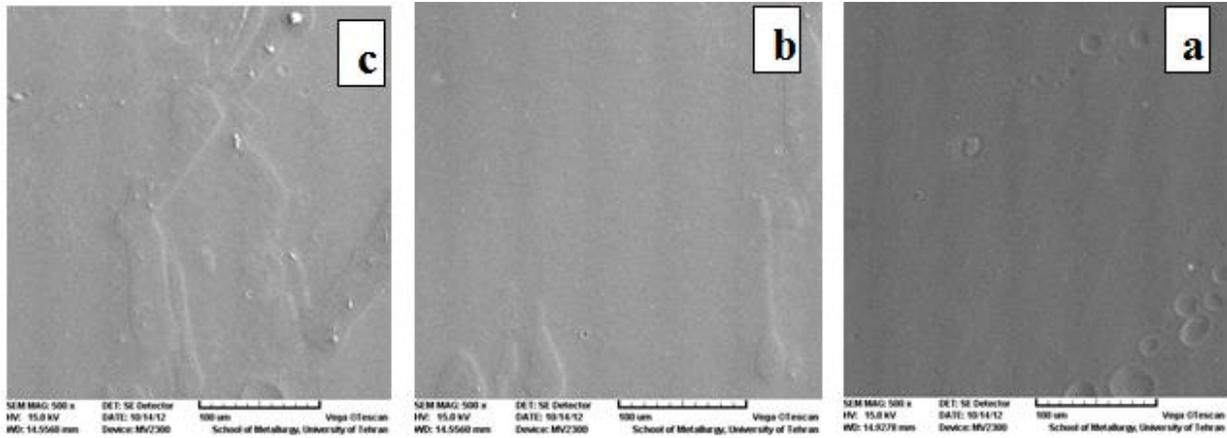


Fig. 5. Scanning electron microscope (SEM) from the surface area (front surface) (1.5 %w/v + 50 %w/w glycerol, a), (2 %w/v + 50 %w/w glycerol, b), (2.5 %w/v + 50 %w/w glycerol, c) trout myofibril protein films.

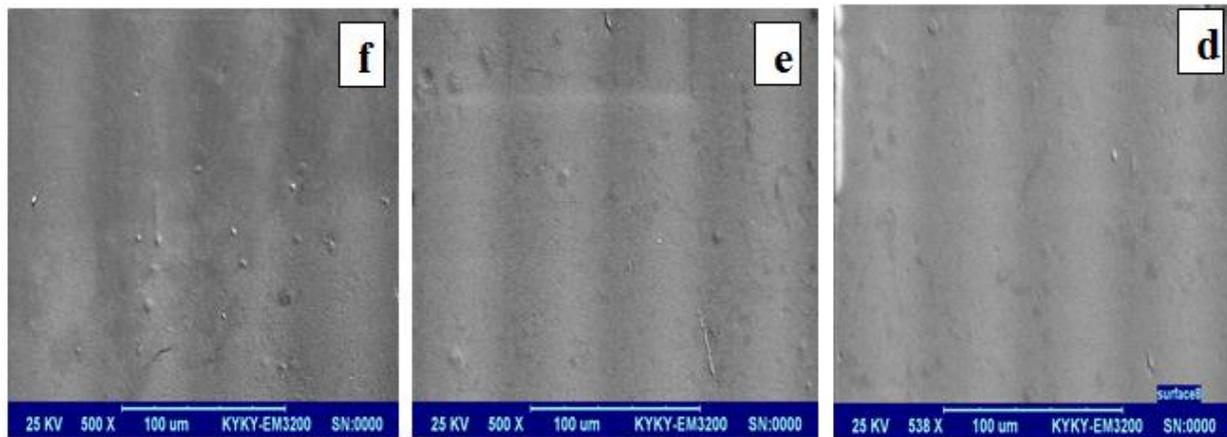


Fig. 6. Scanning electron microscope from the back surface (1.5 %w/v + 50 %w/w glycerol, d), (2 %w/v + 50 %w/w glycerol, e), (2.5 %w/v + 50 %w/w glycerol, f) trout myofibril protein films.

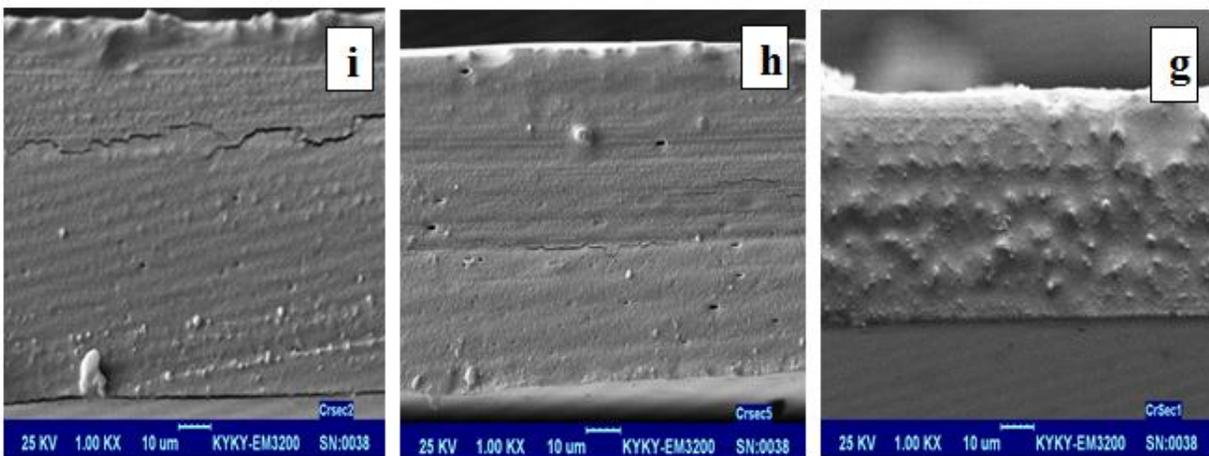


Fig. 7. Scanning electron microscope cross section (1.5 %w/v + 50 %w/w glycerol, g), (2 %w/v + 50 %w/w glycerol, h), (2.5 %w/v + 50 %w/w glycerol, i) trout myofibril protein films.

#### 4. Discussion

For macromolecular network and frequent reaction types, these changes can be as increase in numbers of protein bonds per surface unit that leads to increase of intermolecular reactions numbers.

By thickness increase as the result of dense chains of matrix polymer and decrease of space between chains for water molecules crossing, generally WVP should increase but in protein films due to increase of polar and hydrophilic groups on surface, effect of chains' density increase on WVP decrease is not significant and it will increase because of thickness increase. Investigation into effect of glycerol concentration on protein film thickness of fish showed that by increasing of glycerol content in all dry material content, thickness increases significantly ( $p < 0.05$ ) (Table 1). Because of hydrophilic nature of glycerol from 25% (w/v) to 75% (w/v) moisture value increases and polymer matrix chains stay in further interval so the samples swelling and thickness increases as well (Table 1). The effect of glycerol on thickness of other edible films demonstrates the similar result.

Also, due to disability of natural light to cross through the compact crystal structure, existence of crystal area in a material causes its opacity, this increase of solid material from 1.5% (w/v) to 2.5% (w/v) causes the significant decrease in films' transparency ( $p < 0.05$ ).

Although the glycerol content in this research didn't influence the transparency value ( $p > 0.05$ ), in other researches according to structure and nature of edible film, glycerol addition causes the increase (Mu, 2012) or decrease (Zhang, 2006) of edible film's transparency value.

Regardless the type and concentration of plasticizer, myofibril protein won't spread in water after 24 h and remains untouched in water. Increase in content of utilized plasticizer (regardless of its type), increase the content of soluble dry material in water and causes the linear increase in soluble dry material contents. In fact it is the effect of hydrophilic plasticizer contents change that causes the increase of protein film solubility in water.

Change in non-protein dry material content (in other words) in film as a function of plasticizer concentration causes change in fish dry material solution contents in water. On the other hand due to formation of low weight protein small chains (such as monomers and small peptide) during the film forming solution preservation and being placed in protein matrix could form soluble material in water (CUQ et al., 1995). So, along with the increase of protein content from 1.5 to 2.5%, the sample's solubility in water increases as well (Cuq, 1997). On the whole, plasticizer forms a large proportion of soluble dry material in water. On the other hand, protein network won't spread or solve in water. Corresponding effect of high density and certain presence of intermolecular covalence bonds or physical ties (chains interaction) partially is the reason of the film's insolubility. This behavior of solubility in water can't be generalized and understanding of film solubility in water is so complicated.

Probably high solubility of films can be explained by poor structure of films which contains less TS and more EAB.

Generally, along with glycerol concentration increase, observation of tensile strength value and the length increase in breaking point is a common behavior in case of protein-based films (do A Sobral et al., 2005). Film's tensile strength which is dependent to its thickness has been studied by Genadios to compare the film features with various thickness and different reactions (like plasticizer addition) (Gennadios, 2002). Mahmood and Savlov also suggested that power at the time of whey protein-based film's

breakage, depends on film thickness and plasticizer concentration. Obtained film from fish myofibril protein like other protein film without adding softener are crispy and fragile and have poor and limited functional features.

Film from myofibril protein without plasticizer compare to films that contains plasticizer, shows different mechanical behavior. Considering the obtained results from initial studies myofibril proteins rich in non-ionize polar Amino acids (27mol/100mol amino acid) that are able to form enormous hydrogen protein-protein bonds which causes low adhesion and flexibility of film without plasticizer.

Glycerol is a hydrophilic molecule with low molecular weight that can easily connect the protein chains and establishes hydrogen bond with existing reactive group. When protein and plasticizer stays beside each other, stimulate the creation of plasticizer-protein reactions that causes the destruction of protein-protein reactions. As the result intermolecular reaction density decreases and free space between polymer chains will increase. When the blank films (without plasticizer) are changed, pressure increases continually until the sample breaks. Thus, these films are fragile materials with tensile feature 0.15 mm and high mechanical strength 5.1 N. When plasticizer is added to the sample, experimental non-linear graph, demonstrates the viscoelastic behavior of these films. In addition, by plasticizer concentration increase from 25% (w/w) to 50% (w/w), we observe the decrease of mechanical strength and increase of tensile feature (transformation in breaking point).

Because of the small size of glycerol concentration, placement inside the matrix and reaction with polymer network is easily possible. Thus as glycerol concentration increases 50% (w/w) to 75% (w/w) numbers of glycerol molecule in film surface unit that place between protein chains, increases, as the result density of intermolecular reactions decreases, effective connection between protein chains is low and free spaces of polymer chain decreases. All of these reasons together cause that along glycerol concentration mechanical strength value reduces and elastic features of film increases.

Increase in the number of intermolecular reactions which is related to the film thickness, has no significant influence on length increase up to the film breaking, and has a poor influence on viscoelastic features of this kind of films. In contrary to pressure in time of breaking, thickness increase doesn't have significant influence on tensile increase changes in breaking point (CUQ, 2006).

Plasticizers are considered as materials that due to glycerol concentration increase and containing hydrophilic polar groups, causes the absorption of existing water in circumstance and increase of film's final humidity and decrease of protein-protein reactions which leads to increase of protein chain's movement. This reason, also causes the less resistance and more Hemoelasticity of protein films and in proportion to increase of glycerol concentration from 25% (w/w) to 75% (w/w) value of water vapor penetrability increases (do A Sobral et al., 2005).

In protein films, through increase of protein concentration in film forming solution, because of hydrophilic groups increase, value of water absorption from surrounding circumstance increases which shows its effect in final film thickness (Table 1). Increase of hydrophilic groups leads to chains mobility in polymer matrix and as a result rate of water tendency increases and WVP will increase (Table 2). To form a proper film, the film forming solution should be entirely homogenized and contains low viscosity. By increase of protein concentration from 1.5% (w/v) to 2.5% (w/v) the film forming solution spreading on plate hardly happens and the final

film density reduces. Electron microscope images shows the crack formation and holes increase which is the result of film density reduction along with increase of film forming dry material that causes the WVP increase due to protein concentration in the film forming solution. Effect of glycerol concentration increase on edible film penetrability from primary resource is investigated in other similar research and same results are obtained. Glycerol is a hydrophilic micro molecular softener that causes the decrease of gravity and increase of mobility between polymer chains, moreover by increase of glycerol due to polar groups number increase (OH) hydrogen bonds formation ability with water increases and in turn causes the WVP increases (Chinabhark et al., 2007; CUQ et al., 1995). In order to compare with other functional features (except breakage pressure) that remained constant as a function of film thickness and demonstrated that intermolecular reaction in matrix had been constant. This is a positive deviation of ideal graphs, considering the thickness, and shows that existing hydrophilic material in film tend to humidity which is not considered in PHIC and Henry law.

Similar behavior in protein-based edible films, cellulose and lipid is reported by different researchers (Hagenmaier, 1992). Water transfer modeling among hydrophilic films are greatly complicated due to Isotherm of non-linear water absorption and water content depended circulate (Schwartzberg, 1986). Thus, along with the increase of protein dry material from 1.5% (w/v) to 2.5% (w/v), because initial material participant in Mailard reaction increases, yellowness will be more and lightness will be less. These results are in agreement with and b parameters data and shows that by increase of existing dry material in film, b parameters were increased significantly ( $p < 0.05$ ).

Electron microscope Images of front and back (dorsal) surface shows that films containing the least content of dry material (Fig. 5) in compare to those containing the most have more homogenous surface, because increase of dry material causes a heterogeneous surface that can be proposed as a probable reason to phase separation. By increase of protein film dry material content from 1.5% (w/v) to 2% (w/v), produced film becomes denser and rate of the film tensile and tensile strength increases, however due to decrease of dry material solubility, small cracks form in film cross structure that leads to the film's WVP. But by increase of dry material content from 2% (w/v) to 2.5% (w/v) heterogeneous surface increases and in addition to observable cracks on cross section, there are some small holes in surface structure that leads to WVP increase and decrease of mechanical strength and film tensile capability of films 2.5% (w/v) in compare to the films with less dry material. Main reason of the film's surface cracks presence are high increase of dry material, increase of film thickness (Table 1), lack of polymer structure chains cohesion and lack of adhesive structure formation. Effect of structural adhesion in protein film on the film functional features has been investigated in similar researches and same results have been obtained (Andreuccetti, 2009).

## 5. Conclusion

Obtained experimental results from the present research shows that film's functional features from fish myofibril protein is under the influence of glycerol and dry material concentration but does not influence all the film features. Increase of glycerol concentration causes the color sample's reduction.

On the other hand, more dry material content causes high opacity of samples in compare to the samples with less glycerol. Furthermore, it is possible to conclude that films with higher

protein concentration, in compare to those which contains less protein content, shows more tensile strength but this behavior can be affected by plasticizer concentration. However, all the solution viscoelastic features won't be affected by the protein concentration.

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