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## Journal

## Investigation of biocomposite film properties based on *Saragassum heris* brown algae and *Plantago psyllium* hydrocolloids

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### ABSTRACT

Biodegradable monomers are utilized to produce biodegradable polymers. Today, biopolymers are used as substitutes for conventional packaging in food packaging. The aim of this research is to produce biodegradable biocomposite films based on alginate extracted from the brown seaweed *Sargassum heris* (1.5%, 2%, 2.5%) and *Plantago psyllium* hydrocolloid (0%, 1%, 0.5%, 0.75%), and to investigate the effects of these variables on the physical, chemical, and mechanical properties of the films. The results showed that by enhancing the percentage of PPH and plasticizer increased the moisture, solubility, permeability, and thickness of the film. Scanning electron microscopy (SEM) images showed by increasing the PPH amounts decreased the films uniformity. In Fourier transform infrared spectroscopy (FT-IR), were observed the peaks in 1654 and 1590 cm<sup>-1</sup> for C-O group, 1735 cm<sup>-1</sup> for C=O, 2920 cm<sup>-1</sup> for CH<sub>2</sub> group, 2932 cm<sup>-1</sup> for CH group and 3400 cm<sup>-1</sup> for OH group. when were increased the plasticizer and PPH, decreased the transparency and whiteness of the film and increased the yellow, green, and saturated colors.

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### 1. Introduction

Food packaging and coatings, due to their barrier and protective properties, can significantly contribute to preserving food quality.

The increasing consumption of plastics and petroleum-based composites has led to widespread environmental challenges. Consequently, finding suitable alternatives with high biodegradability and environmental compatibility is proposed as a desirable solution.

The advantages of utilizing biopolymers include their biodegradability in the environment, environmental sustainability, creation of new markets for agricultural products, the ability to control the permeability of water vapor, oxygen, and fat in food, and prevention of the reduction of sensory quality (taste and flavor) (38).

Among the wide range of biopolymers used in the production of multilayer films, polysaccharides are recognized as the primary components due to their abundance and non-toxic nature. These biopolymers typically possess adequate

mechanical strength, moderate physical properties, and importantly, they are edible and easily biodegradable. However, their brittleness and high hydrophilicity are considered limitations.

Starch, cellulose, chitosan, agar, alginate, and carrageenan are among the most widely used polysaccharides in the food packaging industry. Bioactive compounds extracted from marine plants, including sulfated polysaccharides, phlorotannins, and diterpenes, have found widespread applications in various industries due to their antibacterial, antiviral, and anticancer properties (20).

Alginate or alginic acid is a viscous polysaccharide abundantly found in the cell wall of brown algae. Despite being insoluble in water, it has a high water absorption capacity (17). Sodium alginate, extracted from brown algae (Phaeophyceae), is the sodium salt of alginic acid and is a hydrophilic carbohydrate. The primary structural units of this polysaccharide are beta-D-mannuronic acid and alpha-L-guluronic acid. Sodium alginate is used as an emulsifier, stabilizer, and thickener in various industries (4).

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This compound is completely water-soluble and results in the preservation of the sensory properties of the product, including odor, flavor, taste, and color. Additionally, this compound increases the added value and nutritional value of the product by preserving vitamins and essential amino acids and preventing enzymatic activity, thereby reducing waste (2, 13, 36).

Alginate, due to its valuable properties such as non-toxicity, biodegradability, biocompatibility, and low cost, has been considered an attractive material for the production of edible films (43,45). The functional properties of alginate, including thickening, stabilizing, suspending, film-forming, gel-forming, and emulsifying, have been extensively studied (25). The linear structure of alginate enables the formation of strong films with a suitable fibrous network in the solid state, making it a promising film-forming material (8). Alginates have a wide range of applications. These compounds are used in the food industry to reduce dehydration in meat, fish, and fruits (16). They are also used as gel-forming components and colloidal stabilizers in the beverage industry (23). The genus *Plantago*, belonging to the Plantaginaceae family, comprises approximately 250 plant species. This genus has a global distribution, and two significant species are commonly known as *Ispaghul* in Iran, which have extensive applications in industries and pharmaceuticals. The seeds of these plants contain a substantial amount of hemicellulose, consisting of a xylan backbone to which arabinose, rhamnose, and galacturonic acid units are attached. This complex polymeric structure is termed arabinoxylan. Phytochemical studies on various plant species have revealed their high potential for producing a wide range of bioactive secondary metabolites such as iridoids, phenols, polysaccharides, sterols, and alkaloids. Investigations into the plant components of *Plantago ovata* (*Psyllium*) seed husks and comatins have shown that these compounds are used as dietary supplements and medicines for the treatment of diseases (15). Researchers have successfully fabricated various biofilms using a wide range of natural and biodegradable polymers. These polymers include starch extracted from plant sources (27, 29), chitosan (41), polylactic acid (7, 47), agar (6, 9,18, 21), and combinations of agar with other biopolymers (22, 31) Numerous studies have been conducted on the production of edible films. investigated the antibacterial properties of *psyllium mucilage* and thyme extract on the storage of carrots. The results showed that the use of natural compounds from medicinal plants as edible coatings for fresh-cut products resulted in the preservation of visual quality and increased shelf life (4, 30). investigated the effect of edible coatings containing alginate gum and walnut extract on the shelf life of beef fillet. The results showed that these coatings could delay microbial spoilage and oxidation of meat and improve its sensory properties . Moreover produced biodegradable and antimicrobial films based on wheat gluten and evaluated the effect of adding alginate gum and walnut extract on the physicochemical properties of these films. The findings of this study indicated that walnut leaf extract can be used as a natural preservative in combination with food packaging films (19). A biopolymer based on agar extracted

from *Acanthophora* seaweed was developed and its properties were evaluated by Diant et al., 2022 (14).

Results showed that the addition of glycerol to agar increased flexibility, while the addition of PVA polymer increased tensile strength, improved overall physical properties, and decreased water solubility of the agar-based biofilms. Furthermore, the use of these coatings for packaging fruits and vegetables in tropical regions led to an increase in shelf life. The objective of this study is to produce a bio composite film from alginate extracted from *Sargassum angustifolium* brown seaweed and a hydrocolloid (*psyllium* husk) and to evaluate the physical and mechanical properties of the resulting product.

## 2. Materials and methods

### 2.1. preparation of material

#### 2.1.1. Collection *Sargassum heris* brown algae

Brown algae is a rich source of alginate, which is generally found in abundance on spring on the seashore of the Oman sea and the Persian Gulf Coast and due to lack of harvesting and abundant production on the coast, they are found in the tidal region. The *Sargassum heris* was collected from the Persian Gulf Coast in Bandar Abbas province. The collected biomass was washed with seawater and, after removal of sand and epiphytic organisms, transferred to the laboratory. Samples were washed multiple times with tap water to remove any foreign materials or substances. In the final stage, they were washed with distilled water to remove salinity. Subsequently, samples were dried in 50% shade at room temperature (25°C) for 4 days. Samples were stored at -18°C.

#### 2.2.2. Extraction and purification of alginates

Alginate extraction was performed following a modified procedure described by Calumpong et al. (1999)(11). Twenty-five grams of dried algae were immersed in 800 mL of 2% formaldehyde solution for 24 hours at 90°C, washed with distilled water, and subsequently added to 800 mL of 0.2 M HCl and left for an additional 24 hours. Following this incubation, the samples were washed again with Milli-Q water before extraction with 2% sodium carbonate solution for 3 hours at 100°C. The soluble fraction containing alginate was collected by centrifugation at 10,000 g for 30 minutes. Polysaccharides were then precipitated from the supernatant by the addition of three volumes of 95% ethanol. The collected sodium alginate was washed twice with 100 mL of acetone, dried at 65°C, dissolved in 100 mL of Milli-Q water, and finally precipitated again with three volumes of ethanol and dried at 65°C.

#### 2.2.3. Extraction of *P. psyllium hydrocolloid*

Ten grams of *P. psyllium* seeds were subjected to three consecutive 15-minute washes with 96% ethanol

to effectively remove the adhering crust, sand, and other impurities. Subsequently, the seeds were dried at 70°C. The dried seeds were then mixed with 100 mL of distilled water and subjected to extraction at 80°C and 600 rpm to obtain the PPH. The hydrocolloid fraction was subsequently isolated and dried for further use as described in (3).

#### 2.2.4. Production of edible bio composite film

Eleven biocomposite biofilms were prepared utilizing SHA and PPH, as outlined in (34) Following the dissolution of alginate and the P. psyllium-derived hydrocolloid and subsequent cooling to 40°C, varying concentrations of plasticizers (glycerol and sorbitol) were incorporated into the solution containing 60% (w/v) PPH (70% w/w). The mixture was then shaken for 10 minutes, followed by degassing under vacuum. The resulting solution was subsequently cast onto a 20 cm diameter glass plate and dried at 40°C for 24 hours before gentle removal.

**Table.1.** Biocomposite film treatments prepared from SHA and PPH.

Treatment number	SHA	PPH
1	1.5%	0%
2	1.5%	0.5%
3	1.5%	0.75%
4	1.5%	1%
5	2%	0%
6	2%	0.5%
7	2%	0/75%
8	2%	1%
9	2.5%	0%
10	2.5%	0.5%
11	2.5%	0.75%

#### 2.2. Evaluation of bio composite films

##### 2.2.1. Film thickness

Film thickness was measured using a micrometer (Mitutoyo, Japan) with an accuracy of 0.001 inch at 5 random points. These values were averaged to calculate the mechanical properties (tensile strength and permeability to water vapor).

##### 2.2.2. Humidity content measurement

A square piece with a side length of 2 cm was cut from each produced film, weighed, and then placed in an oven at 105 °C for 24 hours (Jeio Tech model OF-12G). The films were weighed again, and the moisture content was calculated using equation 1 (12). Equation 1:

$$\frac{(\text{film weight before heating} - \text{film weight after heating})}{(\text{film weight before heating})} \times 100 = \% \text{ moisture}$$

##### 2.2.3. Film solubility in water

A piece of the prepared film, after weighing, was transferred to distilled water and stirred gently for 24 hours using a shaker at 25 °C. Subsequently, the mixture was filtered using Whatman filter paper. The filter paper, along with the remaining film sample, was then placed in an oven at 105 °C to reach a constant weight. The percentage of solubility of the coatings in water was calculated using equation 2 (33). Equation 2:

$$\text{Percentage of solubility} = \frac{(\text{Weight of dry raw material in the film} - \text{Dry film weight after immersion})}{(\text{Weight of dry raw material in the film})} \times 100$$

##### 2.2.4. Measurement of water vapor permeability

This test was performed according to ASTM E96-95 (1) and used to determine the water vapor permeability of hydrophobic polymer films. In this method, the mass transfer resistance in the gas phase on the film surfaces is negligible. However, in the modified method used for hydrophilic films, the role of partial water vapor pressure in the static air between the film and the cup head is also considered, in addition to the water surface within the cup. It should be noted that in both methods, the measurements are based on gravimetry. However, in this specific method, a layer of film with the same size as the cup head surface was cut, glued to the surface of the cup head, and secured with a clamp. After equilibrating the conditions within the cups, the relationship between weight loss and time was determined over a 24-hour period. Seven consecutive weight measurements were then recorded, and the water vapor permeability of the biocomposite films was calculated using equation 3. Equation 3:

$$WVTR = \frac{\left(\frac{G}{t}\right)}{A} \text{ g/h m}^2$$

$$WVP = \frac{WVTR \times d}{\Delta p} \text{ g mm/kPa h m}^2$$

That G = Film weight difference before and after test, t = test time (h), A =  $\pi r^2$  Cross section = (m<sup>2</sup>) d = Film thickness (mm),  $\Delta p$  = Inside and outside pressure difference of cup (kpa)

##### 2.2.5. Measurement of mechanical properties of film texture

Mechanical properties of films such as stretching strength (TS), elongation percentage (%E) and elastic modulus (EM) was measured according to ASTM 882D standard method (histometer Instron 5566). A piece of 1 x 10 cm was cut from each biofilm and placed on the device with a gap of 50 mm in constant environmental conditions. The distance and force during the expansion of the samples were recorded at 50 mm/min to the breaking point and TS (MPa) and % E (mm) were calculated from equations 4 and 5.

Equation 4:

Stretching strength = (Maximum force input)/ (Surface section film)

Equation 5:

Percentage of elongation = (The amount of stretch until the moment of rupture)/ (primary distance between two jaws)

### 2.2.6. Microstructure

The structure of the selected films was investigated using a scanning electron microscope (SEM, Leo 440i). The samples were placed on aluminum strips mounted on a stainless steel base and then coated with gold for 3 minutes using a sputter coater. Sample surface imaging was performed with an accelerating voltage of 20 kV ( 22).

### 2.2.7. Fourier transform infrared spectroscopy (FTIR) experiment

"Fourier Transform Infrared (FT-IR) spectroscopy was performed on the films to determine their spectral behavior within the frequency range of 400 cm<sup>-1</sup> to 4000 cm<sup>-1</sup> with a spectral resolution of 4 cm<sup>-1</sup>, in accordance with ASTM standard 5477-93."

### 2.2.8. Films color

Colorimetric analysis of the films was conducted using a HunterLab Ultrascan VIS colorimeter. This analysis involved the determination of color parameters, including a\* (red-green), b\* (blue-yellow), and L\* (lightness). Furthermore, the pale yellow color (C), yellow index (YI), and white index (WI) were calculated using equations 6, 7, and 8, respectively (35, 32)

Equation 6:

$$C = \sqrt{a^*^2 + b^*^2}$$

Equation 7:

$$YI = \frac{142.86b^*}{L^*}$$

Equation 8:

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^*^2 + b^*^2}$$

### 2.3. Data analysis

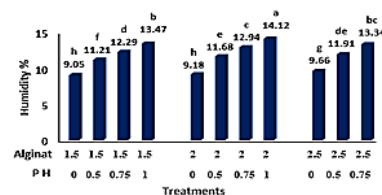
Statistical analysis of the data was conducted using SPSS software (version 19). Levene's test was employed to assess the homogeneity of variances. In instances where homogeneity of variance was not met, analysis of variance (ANOVA) was performed. Subsequently, if a significant difference between group means was detected, the Duncan's multiple range test was utilized for post-hoc pairwise comparisons.

## 3. Results and Discussion

### 3.1. water solubility & moisture content of film

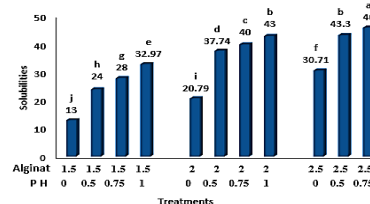
One of the most crucial properties of biodegradable and edible films is their water resistance, as this characteristic directly influences their efficacy in protecting high-water-activity foods or frozen products (28, 42). Conversely, the degree of water solubility can significantly impact the release kinetics of incorporated active compounds, such as antioxidants and antimicrobial agents, from the film matrix (26).

Pure alginate films exhibited relatively low moisture absorption. However, films formulated with a combination of alginate and psyllium seed gum demonstrated significantly higher moisture uptake (24). This trend can be attributed to the increased glycerol content necessitated by the higher concentrations of both alginate and psyllium seed gum within the film matrix. Consequently, as the proportions of alginate and psyllium seed gum increased, so too did the moisture absorption capacity of the films( 39, 46). Statistical analysis revealed a significant difference in moisture absorption between the pure alginate films and those containing the alginate-psyllium seed gum blend. Pure alginate films exhibited rapid dissolution upon immersion in the solvent. Increasing the alginate concentration resulted in a corresponding increase in dissolution time. Films formulated with a combination of alginate and psyllium seed gum demonstrated a slower dissolution rate as the proportions of both biopolymers increased. However, all films, regardless of composition, dissolved completely within 30 minutes. Statistical analysis revealed significant differences in dissolution time among the various film formulations ( $P \leq 0.05$ ).



**Fig.1.** Moisture content of films prepared from SHA and PPH. The letters with the same name show no significant difference and the letters with different name show a significant difference.

A comparative analysis of pure alginate films revealed a positive correlation between alginate concentration and dissolution rate. This observation can be attributed to the hydrophilic nature of alginate, a polysaccharide rich in hydroxyl groups (44). As the concentration of alginate increases, the number of available hydroxyl groups for hydrogen bonding with water molecules also increases, facilitating dissolution. Consequently, films with higher alginate concentrations generally exhibit faster dissolution rates.



**Fig.2.** Films solubility that was prepared from SHA and PPH. The letters with the same name show no significant difference and the letters with different name show a significant difference

3.2. Water vapor permeability (WVP) measurement

Low water vapor permeability is a critical physical property in food packaging design. The results of the water vapor permeability test are presented in Figure 3. Numerous studies have reported that the incorporation of gums into the matrix of other biopolymers, such as proteins and polysaccharides, can effectively reduce water vapor permeability (10,5). Pure alginate films containing 1.5%, 2%, and 2.5% alginate, plasticized with a 60% (w/v) blend of glycerol and sorbitol, exhibited a gradual increase in permeability with increasing alginate and plasticizer content. However, composite films composed of 1.5%, 2%, and 2.5% alginate and 0.5%, 0.75%, and 1% psyllium seed gum, plasticized with a 60% (w/w) blend of glycerol and sorbitol (relative to alginate weight) and a 70% (w/w) blend (relative to psyllium seed gum weight), demonstrated a significantly higher permeability compared to the pure alginate films. It is important to note that all observed differences in permeability were statistically significant.

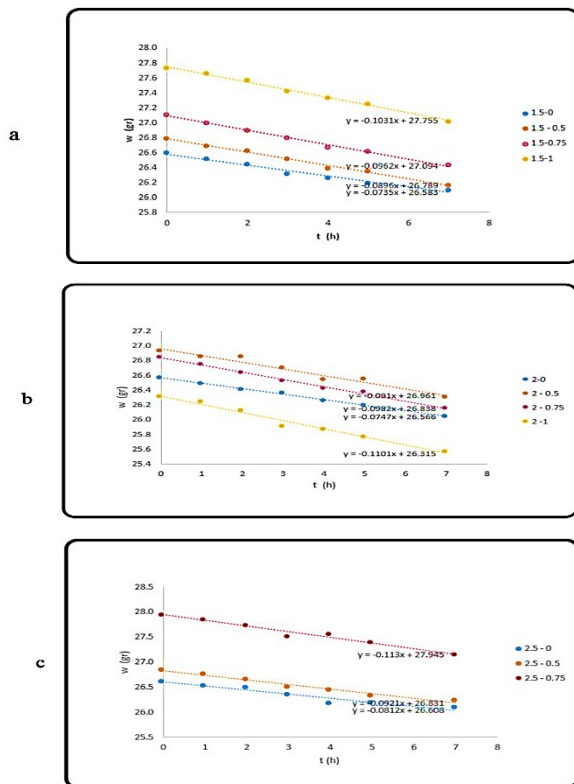


Fig.3. Slope in terms of weight to the time of the prepared films from SHA and PPH

- A. Film containing 1.5% SHA and 0% PPH with blue color, 1.5% SHA and 0.5% PPH with orange color, 1.5% SHA and 0.5% PPH with purple color, 1.5% SHA and 1% PPH yellow color.
- B. Film containing 2% SHA and 0% PPH with blue color, 2% SHA and 0.5% PPH of orange color 2% SHA with 0.75% PPH of purple color, 2% SHA and 1% PPH with yellow color
- C. Film containing 2.5% SHA and 0% PPH of blue color, 2.5% of SHA and 0.5% of PPH orange color, 2.5% SHA, and 0.75% of PPH purple color

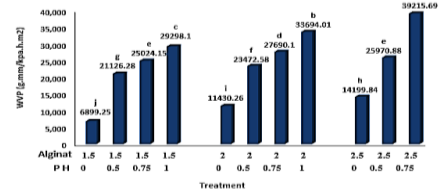


Fig.4. Water vapor permeability of films prepared from SHA and PPH

3.3. Film thickness

Thickness is a critical parameter influencing the physicochemical properties of nanocomposites. Pure alginate films exhibited lower thicknesses compared to those formulated with a blend of alginate and psyllium seed gum. To enhance the flexibility of the films, it was necessary to incorporate plasticizers such as sorbitol and, more significantly, glycerol. It can be inferred that films with higher glycerol concentrations absorbed more moisture, leading to swelling and consequently a substantial increase in film thickness. Biofilms composed of pure alginate (0-2%) and treated with (0-0.5%) exhibited significant differences ( $p \leq 0.05$ ). However, no significant increase in biofilm thickness was observed in treatments containing both alginate and psyllium seed gum ( $p \geq 0.05$ ). Pure alginate biofilms demonstrated similar thicknesses, and this similarity was also observed in other treatment groups (Figure 5). Pure alginate films were observed to be thinner compared to those formulated with a blend of alginate and psyllium seed gum. To enhance the flexibility of the films, it was necessary to incorporate plasticizers such as sorbitol and, more significantly, glycerol. It can be inferred that films with higher glycerol concentrations absorbed more moisture, leading to swelling and consequently a substantial increase in film thickness. In 2012, Ahmadi et al. conducted a study on the impact of various plasticizer concentrations on films based on psyllium mucilage and obtained similar results (3).

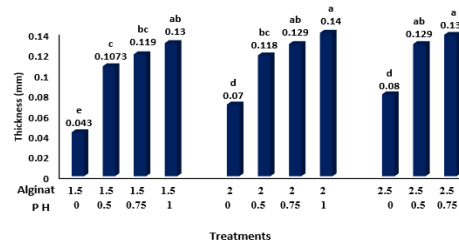


Fig.5. The films Thickness prepared from SHA and PPH The letters with the same name show no significant difference and the letters with different name show a significant difference.

3.4. Mechanical properties

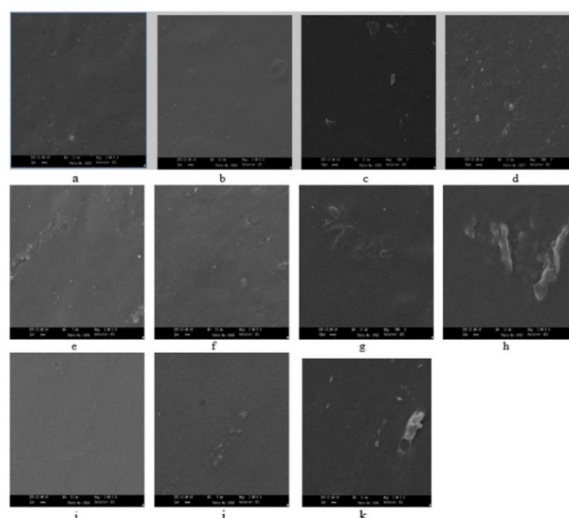
The mechanical characteristics of packaging films are commonly quantified by indices including tensile strength, elongation at break (a measure of flexibility), and hardness. The incorporation of increased glycerol levels into

polysaccharide films facilitates the diffusion of small glycerol molecules into the polymer matrix, thereby diminishing interchain associations and consequently modifying the mechanical attributes of these films. This phenomenon induces a notable enhancement in both the orientational and segmental mobility of polymer chains. Nonetheless, it is imperative to consider that the optimal glycerol concentration for film formation should be within the 25-75% (w/w) range relative to the polysaccharide weight. Glycerol concentrations below the 25% (w/w) threshold yield brittle films, whereas concentrations exceeding 75% (w/w) produce films that are flexible yet adhesive (12). The incorporation of higher glycerol levels led to an increased elongation at break in the relaxed state of the films, a concomitant decrease in tensile strength, and a notable improvement in their overall flexibility. Numerous studies have demonstrated that the fabrication of composite films by combining protein and polysaccharide matrices significantly improves their overall mechanical performance (37,40,48).

### 3.5. Scanning electron microscope (SEM)

A uniform mixture of psyllium husk and alginate could not be achieved. In films with a high percentage of psyllium husk, the solution was relatively heterogeneous, with very small film fragments visible to the naked eye within the film. Increasing temperature and adding plasticizer did not affect the viscosity or homogenize the solution. Consequently, the resulting film was non-uniform in some areas. Additionally, psyllium husk absorbed a significant amount of water compared to alginate, resulting in a significant loss of moisture during drying. Consequently, voids formed in the film instead of water. It is important to note that high temperatures were avoided during film drying to prevent rapid and excessive evaporation of the film, which could lead to defects such as micropores, non-homogeneity, turbidity, and yellowing of the film." Scanning electron microscopy images revealed a highly uniform structure in pure alginate films. However, as the concentration of psyllium husk increased, a decrease in film uniformity was observed. At high concentrations of psyllium husk, distinct fragments of the husk were discernible within the film matrix." The results of the microstructure study of biofilms are presented in Figure 8. The films that used the SHA in the structure (Figures a,e,i) were uniform and the films containing a high percentage of PPH were heterogeneous (Figures c, d, g, h, k).

Increasing the temperature and adding plasticizer had no effect on modulating the viscosity and homogenizing the solution, resulting in a non-uniform film in some areas. Furthermore, PPH absorbed significantly more water in the solution compared to SHA, leading to substantial moisture loss during drying and consequently, the formation of empty spaces within the film instead of water. Importantly, high temperatures were avoided during film drying to prevent rapid and excessive evaporation, which can lead to defects such as small pores and film heterogeneity.

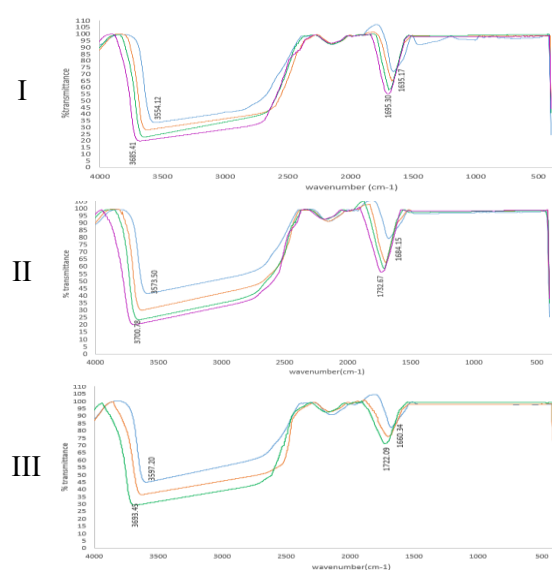


**Fig.8.** Composite biofilm microstructure prepared from SHA and PPH by SEM

- A. The film microstructure containing 1.5% SHA and 0%PPH
- B. The film microstructure containing 1.5% SHA and 0.5%PPH
- C. The film microstructure containing 1.5% SHA and 0.75%PPH
- D. The film microstructure containing 1.5% SHA and 1%PPH
- E. The film microstructure containing 2% SHA and 0% PPH
- F. The film microstructure containing 2% SHA and 0.5% PPH
- G. The film microstructure containing 2% SHA and 0.75%PPH
- H. The film microstructure containing 2.5% SHA and 0% PPH
- I. The film microstructure containing 2.5% SHA and 0.75%PPH

### 3.6. Fourier transform infra-red spectroscopy (FTIR)

When comparing the peaks of pure SHA films with those of SHA and PPH combination films, it was observed that the peak intensity of the SHA and PPH combined films increased. Furthermore, as the percentage of hydrocolloids increased, the peak intensity also increased. These results are presented in Figure 9.



**Fig.9.** Fourier transform infrared spectroscopy of composite biofilms prepared from SHA and PPH

I. Infrared spectroscopy of 1.5% SHA and 0% PPH with blue color, 1.5% SHA and 0.5% PPH with orange color, 1.5% SHA and 0.75% PPH with green color, 1.5% SHA and 1% PPH with purple color.

II. Infrared spectroscopy of 2% SHA and 0% PPH film with blue color, 2% SHA and 0.5% PPH film with orange, 2% SHA and 0.75% PPH film with green, film 2% SHA and 1% PPH with purple color.

III. Infrared spectroscopy of 2.5% SHA and 0% PPH film with blue color, 2.5% SHA and 0.5% orange PPH film, 2.5% SHA and 0.75% PPH film with green color.

In the FTIR spectra of the studied films, two prominent peaks were observed. With increasing concentrations of SHA and PPH, a slight shift in the position of these peaks towards higher wavenumbers was noted. Additionally, the peak intensities decreased with increasing concentrations of SHA and PPH. Specific details are as follows:

Film 0-1.5: Two strong peaks were observed at wavenumbers 3554 cm<sup>-1</sup> with an intensity of 33.85 and 1635 cm<sup>-1</sup> with an intensity of 71.11.

Film 0.5-1.5: Two strong peaks were observed at wavenumbers 3627 cm<sup>-1</sup> with an intensity of 28.02 and 1656 cm<sup>-1</sup> with an intensity of 64.24.

Film 0.75-1.5: Two strong peaks were observed at wavenumbers 3671 cm<sup>-1</sup> with an intensity of 24.05 and 1675 cm<sup>-1</sup> with an intensity of 57.56.

Film 1-1.5: Two strong peaks were observed at wavenumbers 3685 cm<sup>-1</sup> with an intensity of 19.95 and 1695 cm<sup>-1</sup> with an intensity of 55.1534."

Two prominent peaks were observed in the FTIR spectra of the studied films. With increasing concentrations of SHA and PPH, a slight shift in the position of these peaks towards higher wavenumbers was noted. Additionally, the peak intensities decreased with increasing concentrations of SHA and PPH. Specific details are as follows:

Film 0-2: Two strong peaks were observed at wavenumbers 3573 cm<sup>-1</sup> with an intensity of 41.68 and 1684 cm<sup>-1</sup> with an intensity of 80.08.

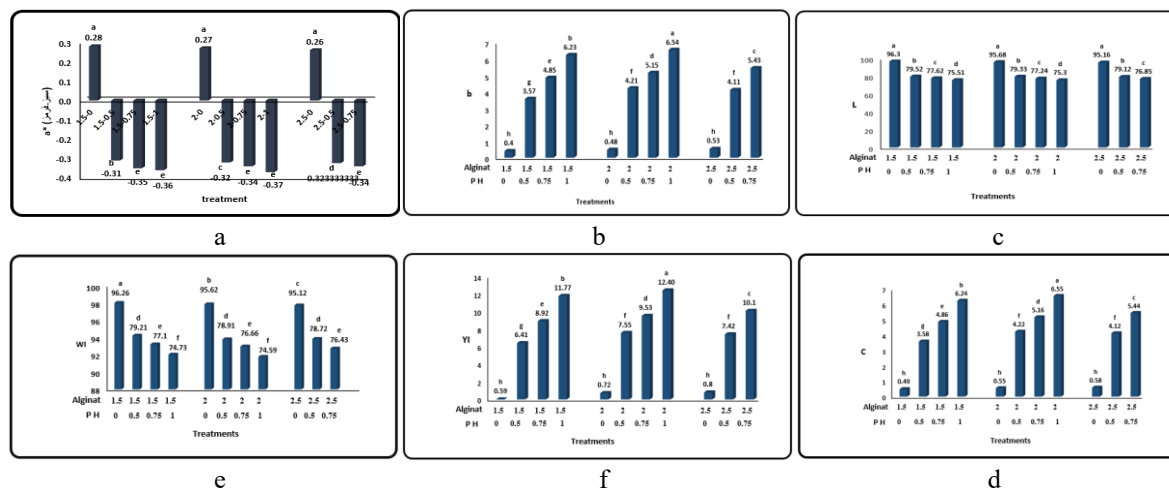
Film 0.5-2: Two strong peaks were observed at wavenumbers 3631 cm<sup>-1</sup> with an intensity of 30.30 and 1689 cm<sup>-1</sup> with an intensity of 62.40.

Film 0.75-2: Two strong peaks were observed at wavenumbers 3676 cm<sup>-1</sup> with an intensity of 24.54 and 1705 cm<sup>-1</sup> with an intensity of 57.93.

Film 1-2: Two strong peaks were observed at wavenumbers 3700 cm<sup>-1</sup> with an intensity of 20.66 and 1732 cm<sup>-1</sup> with an intensity of 56.57

### 3.7. Film color

Color perception, as quantified by L\*, a\*, and b\* color parameters, is a critical determinant of consumer acceptance of edible films. Scientific investigations have demonstrated that variations in glycerol concentration exert a significant influence on the overall color profile of these films. Specifically, in hydrocolloid films based on gum Arabic, an escalating glycerol concentration was associated with an increase in lightness (L\*) and blue-yellowness (b\*) values, concurrent with a decrease in green-redness (a\*). Furthermore, the yellowness index and whiteness index (WI) exhibited an upward trend, which can be attributed to the plasticizing effect of glycerol (3). The study investigated the combined effects of glycerol and gum arabic on the color properties of films. While both glycerol and gum arabic led to an increase in b\*, YI, and chroma, their effects on the a\* parameter differed. Glycerol caused a decrease in a\*, whereas gum arabic induced a significant increase. Additionally, both additives resulted in a decrease in WI and L\*. These findings suggest that the interplay between glycerol and gum arabic ultimately led to a dominance of red tones within the films.



**Figure 10.** Biofilm color that are prepared from SHA and PPH

- index a\* (green - red +) biofilms prepared from SHA and PPH
- index b\* (blue-yellow +) biofilms prepared from SHA and PPH
- L index (transparency) of biofilms prepared from SHA and PPH
- Saturation of biofilms prepared from SHA and PPH
- Yellowing of biofilms prepared from SHA and PPH
- Whiteness of biofilms prepared from SHA and PPH

A slight inclination towards reddish and yellowish tones was observed in the films, contributing to a darker overall appearance. Nevertheless, all films exhibited exceptional

transparency. Although the similarity of these films to conventional polymeric films in terms of whiteness and transparency may be considered a desirable attribute (34), the optimal film properties are contingent upon the intended

application. For example, films characterized by reduced light transmittance can serve as an effective barrier against the oxidation of lipid-rich foods, rendering them suitable for packaging applications involving fatty foods such as peanuts.

#### 4. Conclusion

Pure alginate films demonstrated a direct correlation between increasing alginate concentration and solution viscosity. This enhanced viscosity resulted in increased water absorption and consequently, a higher moisture content in the final films. Furthermore, plasticizer content in both pure alginate and alginate-psyllium hydrocolloid blend films contributed to elevated moisture levels due to the water-retention properties of glycerol. The optimal glycerol concentration for film formation should be within the range of 25-75% (w/w) based on the weight of the polysaccharide. Glycerol concentrations below 25% (w/w) of the polysaccharide weight result in brittle films, whereas concentrations exceeding 75% (w/w) produce excessively flexible and sticky films. Increasing glycerol content enhances the tensile strain at break of the films and reduces tensile strength, while simultaneously improving overall film flexibility. An increase in glycerol concentration in psyllium hydrocolloid films resulted in higher luminosity ( $L^*$ ) values and decreased red-green color coordinates ( $a^*$ ). Films with high psyllium hydrocolloid percentages exhibited heterogeneity due to the lack of uniform dispersion with the alginate solution, resulting in the formation of small, visually discernible particles within the film matrix. Elevating the temperature and incorporating additional plasticizer did not significantly influence the viscosity of these systems.

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#### 6. Declaration of interest

All authors declare that there is no competing interest.

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