A novel pumpkin seeds protein-pea starch edible film: mechanical, moisture distribution, surface hydrophobicity, UV-barrier properties and potential application

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Keywords: pumpkin seeds protein, pea starch, composite films, mechanical properties, swelling degree, pack oily food

Abstract
The pumpkin seeds protein (PSP) films have good gas barrier properties and elongation at break, but their inferior tensile strength and high moisture adsorption needed to be overcome. There are few reports about modification of PSP films so far. This study was to investigate the effects of different ratios (0:5, 1:4, 1.5:3.5, 2:3, 2.5:2.5 and 5:0) of pea starch (PS)/ pumpkin seeds protein (PSP) on the morphology, mechanical properties, water vapor permeability (WVP), optical properties, hydrophobicity, and oil resistance of blend films. According to the results, the scanning electron micrographs of surface and cross-sections indicated the good biocompatibility between PS and PSP. The tensile strength was enhanced from 5.02 MPa to 16.41 MPa with the incorporation of PS at 40% level, but the elongation at break was decreased meanwhile. The addition of PS enhanced the hydrophobicity and reduced the swelling degree of films. And then, the composite films incorporated with PS were more transparent at visible region. The oil soaking had little effect on the mechanical properties of composite films with low starch content. The addition of starch led to the closer interaction between water molecules and macromolecules in film-forming matrix. In addition, the PS/PSP composite films showed inferior barrier properties of water vapor and ultraviolet light compared with pure PSP films. Based on the above various factors, the composite film composed of PS and PSP in a suitable proportion has the potential to package oily foods.

1. Introduction
Currently, a large number of applications of non-degradable petroleum-based materials pose a great threat to environmental safety [1]. Developing affordable biodegradable films and promoting its various functional performances for available packages is still a task to be accomplished in the future. Nowadays, renewable biological macromolecules are increasingly used in research and development of degradable food packaging. They mainly include polysaccharides (starch, chitosan, cellulose and fibers from edible fungi as well as vegetable), proteins (gelatin, collagen, legume protein, zein, etc), lipids (waxes, acetoglycerides and resins) or combination of them [2–4]. To a certain extent, they have great potential to reduce environmental strain and save the oil crisis brought by traditional plastic waste [5]. Therein, protein is a diverse combination of 20 amino acids in different proportions. The advanced structures of proteins determine their chemical bonding and interaction forces at different locations and with different types and energies, which are critical for film formation. The presence of a large number of hydroxyl groups in proteins can help protein to form a gel in water and contribute to film formation [6, 7]. The protein is denatured under extreme conditions such as high temperature, acid and alkali. The protein’s tertiary and quaternary structures are destroyed after denaturation, and the resulting protein chains interact with hydrogen bonds, hydrophobic bonds, covalent bonds, and ionic bonds to give excellent mechanical strength and barrier properties to the protein film [1, 7, 8]. Pumpkin seeds are mainly used for pumpkin oil production in Austria, Hungary, Slovenia and Serbia [9]. After oil extraction,
the pumpkin seed meal with a protein content of about 55% has been used as a by-product mainly for animal feed or directly discarded [10]. The pumpkin seed protein film is stretchy and has the advantage of blocking gas, which gives it the potential as a stretchable gas barrier film. But its optimal tensile strength value is about 6 MPa. It is much lower than that of gelatin crosslinked film and zein film [11–13]. Inferior tensile strength is a typical drawback of protein films. Excellent mechanical properties play a key role in maintaining continuous and non-breakable structure of the material and promoting its barrier properties consequently [14]. What’s more, the hydrophilicity of proteins badly restricts the practical applicability of protein materials. When the film is in high humidity or directly in contact with water, the mechanical strength and barrier properties of protein films will deteriorate due to swelling of water absorption [2, 15]. There are few reports about modification of pumpkin oil cake protein isolate films so far.

Prior studies have found that the addition of polysaccharides could improve the tensile strength of the protein film. Yang et al (2015) showed that the TS of the casein/hsian-tsa gum blend films was significantly higher than casein film. The FTIR results showed that there were hydrogen bonds between casein and hsian-tsa gum, and the Maillard reaction occurred between them, forming a denser structure as compared to pure casein film [16]. As stated by Abugoch et al (2011), the increase of chitosan content in mixture significantly increased the mechanical strength of the quinoa protein/chitosan composite films [17]. Pea starch whose amylose content is about 30%–60% and varies by species belongs to high amylose starch [18]. References have reported about the addition of starch with high content of amylose can increase the tensile strength (TS) of composite films and decrease their elongation at break (EAB). Sun et al (2013) pointed that the addition of pea starch with high content of amylose can increase the tensile strength of peanut protein isolate films [19]. Wang et al (2017) reported that the tensile strength value of collagen-starch composite film blended with high amylose and common starch was significantly higher than that of the added amylopectin composite film [13]. Similarly, the addition of PS may improve the properties of PSP films.

In the present work, the main purpose was to analyze the effects of different ratios of PS/PSP on the morphology, mechanical properties, water vapor permeability (WVP), optical properties, thermal stability, hydrophobicity, moisture distribution, and oil resistance of blend films, which may provide some new ideas for improving the key properties of biodegradable films, especially for tensile strength and hydrophobicity.

2. Materials and methods

2.1. Materials
Pumpkin seeds protein (PSP) with over 90% purity was purchased from the Senyuan Biological Science and Technology Co. Ltd (Shanxi, China). Pea starch (PS) (about 40% amylose) was purchased from the STARPRO Starch Co., Ltd (Chengdu, China). Glycerol was purchased from the Aladdin Industrial Corporation (Shanghai, China).

2.2. Preparation of composite films
The composite film-forming solution (10%, w/w) was prepared as follows. The PS and PSP were accurately weighed and mixed at different ratios of 0:5, 1:4, 1:5:3.5, 2:3, 2.5:2.5 and 5:0 (pea starch content was 0%, 20%, 30%, 40%, 50%, 100% respectively). 5 g pumpkin seeds protein, pea starch or pea starch/pumpkin seeds protein mixture was dispersed in 45 g deionized water. Aqueous suspensions were mixed well and adjusted pH 12 with 1 M NaOH (FE20K, Mettler-Toledo, Switzerland). Addition of glycerol to each solution was set at 0.4 g glycerol/g dry basis and the solutions were incubated and stirred at 90 °C, for 20 min (DF-101S, Baojing Electronic Co., Zhengzhou, China). The mixture was cooled to room temperature and placed in an ultrasonic cleaner (SB 25–120, Scientz biotechnology Polytron Technologies Inc., Ningbo, China) to remove air bubbles. Film solutions (about 20 g) were poured into polycrystalline plate (15 cm × 15 cm) evenly and dried at 30 °C for 24 h in air dry oven (GZX-9070MBE, Boxun Industry Co., Shanghai, China). The finished intact films were peeled off and conditioned in temperature&humidity incubator (SPX-150C, Boxun Industry Co., Shanghai, China) at 25 °C and 50% relative humidity for at least 2 days before tests.

2.3. Film thickness
The thickness of films was measured by a hand-held micrometer (Q/ILBN2-2006CH-1-S, Liuling Instrument Factory, Shanghai, China). Ten measurements were randomly taken and averaged for each sample [13].

2.4. Mechanical properties
The mechanical properties include tensile strength (TS) and elongation at break (EAB), which were evaluated by TA.XT.plus Texture Analyzer (TA. XT. Plus, Stable Micro System, UK). The films were cut into strips (2 cm × 7 cm). The strips were then preconditioned at 25 °C and 50% relative humidity for at least 2 days in
temperature& humidity incubator (SPX-150C, Boxun Industry Co., Shanghai, China) [20]. The measurements were carried out at a crosshead speed of 1 mm s⁻¹ and an initial grip separation of 30 mm. Measurements were replicated at least 5 times for each type of film [21]. TS (MPa) and EAB (%) were calculated by equations (1) and (2), respectively.

\[ \text{TS} = \frac{F_{\text{max}}}{A} \]  

(1)

where \( F_{\text{max}}(N) \) is the maximum load, \( A \) (m²) is the cross-sectional area of the strip.

\[ \text{EAB}(\%) = \frac{L - L_0}{L_0} \times 100 \]  

(2)

where \( L_0 \) (cm) is the initial height of film strips; \( L \) (cm) is the increased height before break of film strips.

### 2.5. Water vapor permeability

Each sample of the films to be tested was cut into a film having a size of 5 cm × 5 cm. The thickness of each sample was tested at 5 points at random by a hand-held micrometer and the average value was taken. Prior to testing, the film was equilibrated for 2 days in a constant temperature and humidity chamber (SPX-150C, Boxun Industry Co., Shanghai, China) at 25 °C relative humidity of 50%.

The film was tightly covered in a 5 ml beaker mouth and sealed by molten paraffin. The test cup was prefilled with anhydrous calcium chloride leaving 3 mm at the top of the cup and weighed. This cup was placed in a constant temperature and humidity chamber conditioned at 25 °C and 70% RH (SPX-150C, Boxun Industry Co., Shanghai, China). The assembly was weighted each 12 h, up to 96 h [12]. In each film type, three samples were tested. WVP (g · mm/m² · h · kPa) was calculated by equation (3)

\[ \text{WVP} = \frac{m \times d}{A \times t \times P} \]  

(3)

Where WVP is the water vapor permeability (g mm/m² · h · kPa), \( m \) is the weight increase of the assembly (g), \( d \) is the film thickness (mm), \( A \) is the test area (m²), \( t \) is the test duration (h), and \( P \) is water vapor partial pressure difference between the outside and inside of the assembly (kPa).

### 2.6. Color measurement

The color of the sample surface was measured by a Minolta Chromameter (CIE-LAB, Minolta Camera Co., Osaka, Japan). The \( L^* \), \( a^* \) and \( b^* \) values of a white standard plate were measured firstly. The color values were determined by the average of five measurements at different positions for each sample.

### 2.7. Light barrier properties and transparency

According to the methods published previously [22, 23], the Light barrier properties and transparency of the films were measured at the wavelengths from 200 to 800 nm using a UV-VIS-NIR spectrophotometer (UV-3600, Shimadzu Co., Kyoto, Japan). The transmission at each wavelength of the films was showed by the wavelength scanning. The film transparency was calculated by equation (4).

\[ \text{Transparency} = - \log \frac{T}{x} \]  

(4)

where \( T \) is transmission (%) at each wavelength; \( x \) is film thickness (mm).

### 2.8. Thermal properties

The films were preconditioned at 25 °C and 50% relative humidity for at least 2 days. 3 to 5 mg samples were taken and placed in \( \text{Al}_2\text{O}_3 \) crucible for the TGA measurement (Q50, TA Instrument, USA) with a heating range from 35 °C up to 600 °C at a heating rate of 10 °C min⁻¹, which ran under the protection of nitrogen atmosphere.

About 3 mg samples were taken and placed in hermetic pans for the DSC measurement (DSC60, Shimadzu Co., Japan) with a heating range from 25 °C up to 250 °C at a heating rate of 5 °C min⁻¹. An empty hermetic pan was set as the reference.

### 2.9. Contact angle measurements

Water contact angle measurement (PGX, FIBRO SYSTEM.AB, Sweden) was conducted on a contact angle testing system with a droplet with volume of 4 μl. The water droplets are dropped on the surface of the films, and the contact angle between the water droplet and the film surface was observed by the microscope within 5 s. Five different points on the sample surface were tested [24].
2.10. NMR transverse relaxation time (T2) measurements
According to the methods reported by Li et al. (2017), the NMR transverse relaxation time were measured by a Niumag Pulsed NMR analyzer (MicroMR-23, Niumag Corporation, Shanghai, China). SW was set as 200 KHz, TW was set as 1000 ms, NS was set as 32.

2.11. Oil resistant test
Each sample was cut into a film having a size of 4 cm × 4 cm and placed on the filter paper. 0.5 ml of cooking oil was added into the center of the film. The leakage of cooking oil was observed after one week.
To study the effect of oil on the mechanical properties including tensile strength (TS) and elongation at break of the films, the sample strips (2 cm × 7 cm) were soaked in cooking oil for a week. Before testing, the residual oil was removed by the filter paper.

2.12. FTIR analysis
ATR-FTIR spectroscopy was to provide the structural analysis for the samples, which carried out by using a Thermo Nicolet iS 50 FTIR spectrometer from 4000 to 400 cm⁻¹.

2.13. Scanning electron microscopy (SEM)
The micromorphology including the surface and the cross-section of the films was featured by a scanning electron microscope (SEM) (SU1510, Hitachi Co., Japan) at 15.0 KV. The films were frozen in liquid nitrogen and snapped immediately to prepare for observing the cross-sections images. Then, the samples were coated with gold and observed [25].

2.14. Statistical analysis
All data were presented as mean ± SD (standard deviation) from at least three independent trials. The differences between the mean values of these multiple groups were further analyzed and compared by one-way analysis of variance (ANOVA) with Duncan’s multiple tests. ANOVA data with p < 0.05 were considered as statistical significance. Data was analyzed and reported using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Scanning electron microscopy (SEM)
The scanning electron micrographs of film surface are showed in figure 1. The microscopic surfaces of pure PSP and PS film were flat and homogeneous without cracks or holes, which was consistent with the previous reports of Chen et al. (2009) and Mehyar et al. (2012) [25, 26]. With the increase of starch addition, the roughness of the surface of the composite films gradually increased. Though the composite films had rough surface, the overall structure of films was still compact and had no pores and cavities. And then, there was no obvious boundary between protein and starch granules. The partial gelatinization of PS could be responsible for the formation of compact structure [13]. This result indicated the good biocompatibility between PS and PSP.

The cross sections images of the films are showed in figure 1. It can be observed that each treatment group had a compact and smooth structure without layering or phase separation. According to the explanation of Mali et al. the reason for the above results may be that the presence of PSP and PS did not excessively disrupt the gel structure of the other, and the two ingredients closely interacted to form a uniform network, which is beneficial to the physical integrity and stability of the films macroscopically [27]. Compared with pure PSP films, the cross-sections of composite films had a few lines. With the increase of starch content, the thickness of the composite films gradually increased, and the edge gradually became irregular, which was in agreement with the surface microstructure.

3.2. Film thickness
The thickness of the PS/PSP composite films is showed in table 1. The thickness value of the pure PSP film was significantly lower than that of other experimental groups. With the increase of the content of PS, the composite films got thicker. The pure PS starch film showed the highest thickness value.

As reported previously, when the film was not elastic enough, its structure would be more rigid, so the thickness of the film would increase [28]. PS films had tougher structures than PSP films, so the composite films added with PS were thicker. Similar trend was presented previously for the PS/PPI (pea starch/peanut protein isolate) composite films, suggesting that the incorporation of PPI decreased the films thickness [19]. Furthermore, Wang et al. (2017) reported that the high amylose corn starch/collagen composite films were thicker than pure collagen films [13].
3.3. Mechanical properties

The results of tensile strength (TS) and elongation at break (EAB) of films are showed in Table 1. When the PS content was 40%, the composite film showed the highest TS but the lowest EAB. The pure PSP film showed the

![Figure 1. SEM images of the surfaces (A-F) and cross sections (a-f) of pure pumpkin seeds protein (PSP), pea starch (PS) and PS/PSP composite films. FPS/PSP (0:5), FPS/PSP (1:4), FPS/PSP (1.5:3.5), FPS/PSP (2:3), FPS/PSP (2.5:2.5) and FPS/PSP (5:0) means the mixed ratio of PS/PSP on a dry basis were 0:5, 1:4, 1.5:3.5, 2:3, 2.5:2.5 and 5:0 respectively.](image)

| Film composition | Thickness (µm) | TS (MPa) | EAB (%) | TS* (MPa) | EAB* (%) | WVP (g mm m⁻² h kPa) |
|------------------|--------------|---------|--------|---------|--------|--------------------|
| F₀:₅             | 61.13 ± 5.49<sup>d</sup> | 5.02 ± 0.30<sup>d</sup> | 47.61 ± 3.35<sup>a</sup> | 4.57 ± 0.36<sup>d</sup> | 42.64 ± 3.58<sup>a</sup> | 0.132 ± 0.009<sup>d</sup> |
| F₁:₄             | 79.67 ± 0.99<sup>c</sup> | 8.40 ± 0.61<sup>d</sup> | 22.85 ± 1.42<sup>b</sup> | 6.86 ± 0.35<sup>c</sup> | 26.55 ± 2.28<sup>b</sup> | 0.143 ± 0.007<sup>bc</sup> |
| F₁.₅:₃.₅         | 85.67 ± 4.15<sup>bc</sup> | 11.96 ± 0.68<sup>c</sup> | 21.52 ± 0.78<sup>b</sup> | 9.23 ± 0.76<sup>b</sup> | 23.34 ± 1.82<sup>a</sup> | 0.161 ± 0.009<sup>bc</sup> |
| F₂:₃             | 89.07 ± 4.65<sup>b</sup> | 16.41 ± 0.98<sup>b</sup> | 10.87 ± 0.98<sup>a</sup> | 10.87 ± 0.98<sup>a</sup> | 21.00 ± 1.59<sup>b</sup> | 0.193 ± 0.010<sup>c</sup> |
| F₂:₅:₂:₅         | 89.13 ± 1.52<sup>b</sup> | 14.65 ± 1.14<sup>b</sup> | 7.04 ± 1.09<sup>c</sup> | 11.43 ± 1.39<sup>a</sup> | 22.31 ± 1.22<sup>a</sup> | 0.189 ± 0.013<sup>a</sup> |
| F₅:₀             | 98.53 ± 2.43<sup>b</sup> | 14.42 ± 0.91<sup>b</sup> | 9.49 ± 1.76<sup>c</sup> | 10.31 ± 0.89<sup>c</sup> | 23.01 ± 1.02<sup>a</sup> | 0.204 ± 0.006<sup>b</sup> |

<sup>a-d</sup> Means within each column followed by different superscript letters are significantly different (p < 0.05). Values of Thickness are the means of ten replicates (means ± standard deviation). Values of TS and EAB are the means of five replicates (means ± standard deviation). Values of WVP are the means of three replicates (means ± standard deviation).

5.3. Mechanical properties

The results of tensile strength (TS) and elongation at break (EAB) of films are showed in table 1. When the PS content was 40%, the composite film showed the highest TS but the lowest EAB. The pure PSP film showed the
When high amylose PS was blended with other macromolecular like proteins, it was easy to form an ordered amylose is polymerized by linear macromolecules without branches and has good force between PS and PSP, mainly including electrostatic, hydrogen bond, van der Waals force to 16.41 MPa but the EAB reduced from 47.61% to 6.46%.

As mentioned in the article, the reason for the increase of TS value in the above results may be the interaction force between PS and PSP, mainly including electrostatic, hydrogen bond, van der Waals force [29]. And then, amylose is polymerized by linear macromolecules without branches and has good film-forming properties. When high amylose PS was blended with other macromolecular like proteins, it was easy to form an ordered structure, thereby improving the mechanical strength of the biofilms [30]. The decrease in EAB may be due to the brittle texture of PS film and the tougher structure induced by the crosslink reaction [5]. The greatly increased viscosity of the starch after gelatinization could result in less homogeneous texture and uneven tension of the films. That is why the TS value decreased when the PS content reached to 50% and 100% compared with composite films with starch content of 40%.

### 3.4. Water vapor permeability measurement

The results of water vapor permeability (WVP) of films are showed in Table 1. With the increase of PS contents, the WVP of composite films gradually increased. The WVP of pure PSP film was significantly lower than that of other groups. Moreover, pure PS film showed the highest WVP. The WVP of the composite films with starch content of 20% and 30% was significantly lower than that of the experimental groups with 40% and 50% starch content.

Because most biofilms have strong hydrophilicity, a few studies showed that the WVP value may be related to the thickness of the film. As reported by Gutiérrez et al the greater film thickness often led to greater water vapor transmission for hydrophilic films. They believed that the increase of thickness for the hydrophilic film, the greater the partial pressure of water vapor on the inner surface of the film, which ultimately increases the WVP value of the film [31]. Similarly, this view may be an explanation for the above test results.

### 3.5. Color measurement

The color change of the films with the increase of the PS content is showed in table 2. The L*, a*, and b* values for the standard plate were \( L^* = 92.497, a^* = 0.670, \) and \( b^* = -8.617, \) respectively. According to the results, the pure PSP film had the lowest \( L^* \) value (\( L^* = 73.646 \pm 1.122 \)). As the starch content increased, the composite films gradually were brighter, which is advantageous for the transparency of the composite films in the visible light. In addition, the incorporation of PS led to the decrease of \( b^* \) value. The color of the protein film is mainly related to the type and concentration of the film-forming protein and the amino acid contained therein, and has almost nothing to do with the film formation method or process [5]. In this experiment, the addition of PS reduced the protein content of composite films correspondingly. Therefore, the \( b^* \) value gradually decreased with the increase of PS addition.

### 3.6. Light barrier properties and transparency

Transmission curves in the wavelengths from 200 to 800 nm of all films was showed in figure 2. The transmittance of pure PS film was distinctly higher than that of other groups in the entire wavelength range. It is in good agreement with previous study [25]. For composite films and pure PSP film, these curves had similar trend. With the PS content increased, the transmission of UV light in the range of 200–350 nm increased, which indicated that the addition of PS could deteriorate the UV barrier properties. Even so, the transmittance value of these composite films at 200–350 nm was close to zero, and the UV-barrier properties of the composite film containing protein still was vastly superior to some polysaccharide films that have been reported [21–23]. The
results showed a promising application for the composite films in food packaging to prevent from lipid oxidation, which is likely to owe to the high content of aromatic amino acids in proteins in composite films [32].

And the transparency value (T value) at visible light (600 nm) was showed in table 2. According to the transparency equation, the high T value indicates opacity. the increasing PS content in composite films led to the lower T value, which indicated that the composite films were more transparent at the visible light region. That confirmed that the increase of L* value and decrease of b* value played a great part in making the film more transparent.

3.7. Thermal properties
The thermogravimetric (TG) and Derivative thermogravimetric (DTG) curves are showed in figure 3. There were two weight loss steps for pure PS films. The first one occurred between 50 °C–130 °C (peak temperature: 85 °C), which can be attributed to the loss of bonded water in films [33]. According to the published article, the second one with around 70% of weight loss that occurred between 130 °C–400 °C (peak temperature: 332 °C) was used to evaluate the main degradation process [34]. Except for pure PS film, other groups approximately exhibited three main stages of weight loss. The first weight loss stage was detected as 50 °C–130 °C, probably due to the loss of bonded water adsorbed in the films. The second stage in the range of 150 °C–250 °C gave a maximum degradation rate of around 210 °C may be explained by the degradation of low molecular weight protein constituents in composite films [35]. The third stage, between 250 and 400 °C, was possibly associated to the main degradation of protein for pure PSP films and mixture of protein and starch for composite films. It
should be noted that with the increase of PS content, the maximum weight loss rate in this stage of films got higher, suggesting that the addition of PS could deteriorate the thermal stability of composite films at some extent.

As shown in figure 4, there were two endothermic processes in DSC thermograms for all films. For the first endothermic process, the addition of PS had no regular effect on the endothermic peak temperature. For each composite film and pure PS film, the second endothermic peak occurred at 209.68 °C, 198.04 °C, 204.09 °C, and 202.08 °C, respectively, which were lower than that of pure PSP film (216.79 °C). The endothermic behaviors were mainly related to the dissociation of hydrogen bond and dehydration [3].

### 3.8. Contact angle measurements

The results of dynamic contact angle between the water droplets and the film surface are showed in table 3. The contact angle can characterize the hydrophobicity and water solubility of materials. A certain amount of polar peptides in the protein confers strong hydrophilicity to the protein itself [36]. That is why pure PSP film was too absorbent and consequently has strong swelling properties, and the water contact angle cannot be accurately measured after the first second. Moreover, because the pure PS film was extremely uneven, its water contact angle cannot be accurately measured. The contact angle of films was increased by the addition of PS and lasted up to 5 s, indicating that the addition of PS greatly enhanced the hydrophobicity and reduced the swelling degree of the composite films. The hydrophilic groups contained in the protein are mainly carboxyl groups, amino groups and hydroxyl groups, while in starches are mainly hydroxyl groups. Because the polarity of carboxyl group and amino group is greater than that of hydroxyl groups, the hydrophilicity of protein is greater than that of starch. Therefore, the degree of water absorption and swelling of protein is greater than that of starch.

According to the report, Dong et al showed that the increase in the film surface roughness increases the contact area between the water droplet and the surface of the film, thereby reducing the contact angle [12]. In contrast to...
this result, the matte surface caused by addition of PS failed to led to the obvious decrease of contact angle. Hence, the increase of relatively hydrophobic groups may play a role in improving the hydrophobicity of films. Above all, the films incorporated with PS had the superior water resistance and showed the better morphology, compared with the pure PSP and PS film. These results were similar with a previous report \[37\].

3.9. NMR transverse relaxation time (T2) measurements

Water molecules are divided into several components depending on their molecular fluidity or how well they bind to the larger molecules in the gel. When the value of T2 is less than 10 ms, the water state is bound water and it changes to immobilized water and free water when the value of T2 is between 10 and 100 ms and more than 100 ms, respectively. In general, the decrease in T2 value means the closer interaction between water molecules and biological macromolecules.

As showed in figure 5, there are two components of water for all samples. The first one occurred at 0.50–1.15 ms. The T21 value of pure PSP film was the highest, reaching 1 ms. With the increase of starch content, T21 value of each group of samples gradually decreased. For the second one, with the increase of PS content, the relaxation time significantly decreased from 114.98 ms to 37.65 ms, which indicated that the water molecules contained in the matrix of composite films bind more firmly and become less fluid compared with pure PSP film. Both protein and starch bind to water molecules through a gel network. The addition of starch may enhance the gel network structure of the protein, so the water molecules bind more closely to the macromolecules of the film-forming matrix. The results were similar to the results of Yang et al. They showed that the hsian-tsao gum belonging to polysaccharide, and its addition increases the bonding degree of between water molecules and macromolecular \[16\].

3.10. Oil resistant test

The oxidation of oil plays an important role in the spoilage of oil and oily foods. Photooxidation is one of the main causes of oil rancidity. PSP/PS composite film has a remarkable blocking effect on ultraviolet light. Therefore, it is of practical significance to study the oil resistance of the film.

The TS and EAB values of films soaked in oil were showed in table 1. After immersing in cooking oil for one week, the tensile strength of each group of films decreased in varying degrees. When starch content was 0%, 20%, 30%, the tensile strength of films did not decrease significantly. When the starch content was more than 30%, the mechanical strength of the films was remarkably deteriorated, and the elongation at break was simultaneously increased. Therefore, the oil soaking has little effect on mechanical properties of composite films with low starch content. The results in figure 6 showed that there was no leakage of oil for all samples one week later and the composite film had good heat sealability. Combined with the superior UV-barrier properties, this material can be used to wrap foods with high oil content but low moisture content such as high fat dessert and flavoring bag of convenience foods like figure 6.

3.11. FTIR analysis

The infrared spectrum of the films is depicted in figure 7. The FTIR spectra of PS/PSP composite films were similar to the pure PSP films. For pure PSP films, the absorption band at 3296.31 cm\(^{-1}\) was assigned to the
presence of –OH groups between peptide chains and chains and moisture. Besides, the –CH stretching vibration was at ∼2925.23 cm\(^{-1}\) \([37, 38]\). The absorption bands at 1652.32 cm\(^{-1}\) and 1548.61 cm\(^{-1}\) represented amides I and II, respectively. The amide I (1700–1600 cm\(^{-1}\)) band indicates secondary structure of the protein \([12]\). As the starch content increased, there was no significant shift in the absorption peaks of the amides I and II, so the addition of starch had no significant influence on the secondary structure of the pumpkin seed protein.

As previously reported, the absorption bands at about 1150 cm\(^{-1}\) and 1079 cm\(^{-1}\) were considered to the stretching vibration of C–O–C and C–C skeleton vibration respectively, which are typical functional groups of starch \([13]\). Pure PS film exhibited characteristic peaks at 1152.81 cm\(^{-1}\) and 1078.52 cm\(^{-1}\). Apparently, with the increase of starch content, the intensity of the absorption peak at about 1153 cm\(^{-1}\) and 1079 cm\(^{-1}\) of the composite films gradually enhanced. The absorption band at about 1022 cm\(^{-1}\) corresponds to the typical amorphous region of starch \([39]\), which appeared only in pure PS films.

**Figure 6.** Oil leakage test results (A: 0 day B: after 7 days) of pure pumpkin seeds protein (PSP), pea starch (PS) and PS/PSP composite films and oil packaging (C) test of composite film.

**Figure 7.** FTIR spectra of pure pumpkin seeds protein (PSP), pea starch (PS) and PS/PSP composite films.
4. Conclusion

A novel biodegradable film of PS/ PSP composite film was successfully prepared. This study discovered that the mechanical strength, hydrophobicity, and permeability to visible light of the composite film are improved after the addition of starch. Moreover, the immersion of oil had no significant effect on the mechanical properties of the composite films with low starch content. The significant decrease in relaxation time suggested water molecules contained in the matrix of composite films bind more firmly and become less fluid compared with pure PSP film. At the same time, however, the elongation at break was reduced, and the barrier properties against water vapor and ultraviolet light were deteriorated. Taking into account various factors, the composite film with appropriate starch content had the potential to package oily foods. Finally, according to the results of electron microscopy, the biocompatibility between PS and SPS is very good. In summary, the addition of pea starch had a positive effect on the composite film, indicating that the combination of starch and protein may be considered as a new method to improve the properties of biomaterials.

Acknowledgments

This research was supported by grants-in-aid for the Tianjin technology demonstration project for integrated development of primary, secondary and tertiary industries (project number:17ZXYENCO0010, China) and Gaoyou key research and development projects from Modern Agriculture (project number: GY201812, China).

Conflicts of interest

The authors declare that they have no conflict of interest.

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