

# High-Pressure Homogenization Lowers Water Vapor Permeability of Soybean Protein Isolate–Beeswax Films

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**ABSTRACT:** Soybean-protein isolate (SPI) has excellent film-forming capacity. However, the water vapor permeability of SPI film is high, which will cause the moisture loss of packaged products. The effect of high-pressure homogenization (HPH) on the water vapor permeability of SPI–beeswax films was evaluated. The HPH was effective at lowering the water vapor permeability of SPI–beeswax films to about 50% of the control. The HPH reduced the particle size of films and made their matrix more compact. The HPH improved the hydrophobicity of SPI–beeswax films. For the first time, we proved that the HPH improved the bound-beeswax content in SPI–beeswax films. The bound beeswax was effective at lowering the water vapor permeability of films rather than the free beeswax in the film matrix. In summary, the HPH lowered water vapor permeability of SPI–beeswax films by reducing their particle size and raising their hydrophobicity and bound-beeswax content.

**KEYWORDS:** High-pressure homogenization, water vapor permeability, soybean protein isolate, beeswax, hydrophobicity, bound beeswax

## INTRODUCTION

Edible films are novel packaging materials, being edible, degradable, and reproducible.<sup>1</sup> Soybean protein isolate (SPI) has excellent film-forming capacity among materials.<sup>1,2</sup> However, a traditional SPI film will cause the moisture loss of packaged products, so that their quality is damaged because of their high water vapor permeability (WVP).<sup>2,3</sup>

Optimization of film composition and the development of film processing are two main ways to lower the WVP of films. On the one hand, lipids, such as beeswax, lauric acid, palmitic acid, stearic acid, and oleic acid,<sup>1,2,4–7</sup> cross-linking agents, such as sodium alginate, carboxymethyl cellulose, propylene glycol alginate, ferulic acid, and tannic acid,<sup>8–11</sup> and alkylation agents<sup>9</sup> have been optimized to lower the WVP of protein-based films. Among the components, beeswax is cheap and effective to lower the WVP of films.<sup>1,2,12</sup> On the other hand, the film processing also influences the WVP of films. The alkali treatments,<sup>13</sup> enzymatic treatments,<sup>11,14,15</sup> extrusions,<sup>16</sup> and high hydrostatic pressure treatments<sup>17</sup> change the WVP of protein-based films. Remarkably, high-pressure homogenization (HPH) is a common process to improve the stability of emulsions by reducing their particle size.<sup>18</sup> Thus, the HPH has a potential to reduce the particle size of SPI and beeswax in SPI–beeswax films, so that their WVP could be lowered. However, the effect of the HPH on the WVP of SPI–beeswax films has not been studied to our knowledge.

The aim of this study is to evaluate the effect of the HPH on the WVP of SPI–beeswax films. Moreover, the microstructure, hydrophobicity, and beeswax distribution of SPI–beeswax films were explored to give a deeper insight of WVP improvement. The beeswax distribution in the film matrix was discussed for the first time.

## MATERIALS AND METHODS

**Film Preparation.** Films were prepared according to the preceding works, with a few modifications.<sup>1,12,19</sup> Specifically, SPI (5 g, GS5000,

Gushen Biological Technology Group Co., Ltd., China), glycerol (1.5 mL, Sinopharm Chemical Reagent Co., Ltd., China), and beeswax (0.6 g, Sinopharm Chemical Reagent Co., Ltd., China), which was based on our recent work,<sup>12</sup> were mixed in 100 mL of distilled water. The mixture was magnetically stirred at 90 °C for 30 min to acquire a homogeneous emulsion. The emulsion was subjected to a high-pressure homogenizer (NS101L2K, GEA Niro Soavi S.p.A., Parma, Italy), which was preheated to 60 °C with a pressure of 0.1, 5, 10, 30, 50, 100, and 150 MPa for three cycles. The resultant solution was cast onto plexiglass plates and dried at 36 °C for about 36 h. In succession, the dried films were conditioned at 25 °C and a relative humidity of 45% for further analysis.

The control was SPI–beeswax films without HPH treatment. The SPI films were prepared following the same way as the control, except for the addition of beeswax, which was used to exclude the disturbance in the differential scanning calorimetry (DSC) analysis. The films subjected to the HPH with a pressure of 0.1 MPa were prepared to exclude the disturbance of the flow in the homogenizer.

**Determination of WVP.** WVP of the films was determined by a MOCON tester (model PERMA TRAN-W 1/50G, Minneapolis, MN) at 25 °C according to the American Society for Testing and Materials (ASTM) standard method.<sup>20</sup> The films were placed in a test cell with a 5 cm<sup>2</sup> testing area and exposed to pure N<sub>2</sub> flow on both sides. The relative humidity of the permeate side and dry side was 76 and 15%, respectively. The system measured the amount of water vapor transmitted through the film. WVP was calculated by dividing the water vapor transmission rate by the difference in the relative humidity between the sides of the film (0.1 MPa) and multiplying by the average film thickness.

**Imaging.** The films were imaged using scanning electron microscopy (S-4800, Hitachi Co., Ltd., Tokyo, Japan) without coating.<sup>2</sup> The surface and cross-section of the films were imaged with an acceleration voltage of 5 kV.

**Contact Angle Analysis.** The contact angles of the films were measured on a Krüss G10 contact angle goniometer (Krüss GmbH,

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Hamburg, Germany).<sup>1</sup> The contact angles were an average of six random measurements.

**Determination of Water Adsorption Capacity.** The water adsorption capacity of the film solution was determined by thermogravimetric analysis (TGA).<sup>21</sup> An aliquot of 20 mg of the film solution was weighted in a crucible and transferred to a thermogravimetric analyzer (TGA/DSC 1, Mettler Toledo, Switzerland). The weighted crucible without a lid was heated from 40 to 160 °C at a rate of 5 °C/min with a nitrogen flow of 40 mL/min.

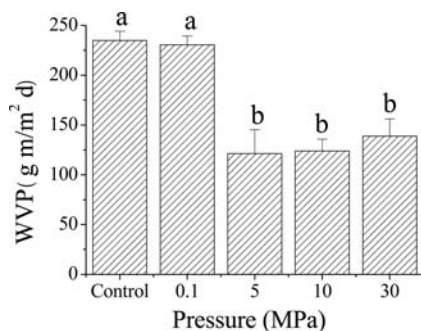
**Determination of Free Beeswax Content.** The content of free beeswax was determined by a DSC analysis.<sup>22</sup> Specifically, the film (about 5.0 mg) was weighted in a crucible and transferred to a preheated calorimeter (Diamond DSC, Perkin-Elmer, Shelton, CT) in a nitrogen bath of 40 mL/min. The sealed crucible was heated from 30 to 90 °C at a rate of 10 °C/min.  $\Delta Y$  in 63.7–67.5 °C was calculated using Pyris software (version 3.8).

A working curve was prepared with beeswax. The beeswax (0–20 mg) was weighted, sealed, and heated from 30 to 90 °C at a rate of 10 °C/min in DSC analysis. The working curve shows the relationship between  $\Delta Y$  and the beeswax content.

**Statistical Analysis.** Analysis of variance (ANOVA) used Microcal Origin 7.5 (Microcal Software, Inc., Northampton, MA). ANOVA tests were carried out for all experimental runs, to determine the significance at the 95% confidence interval. All experiments were conducted in triplicates or more.

## RESULTS AND DISCUSSION

**Effect of the HPH on the WVP of SPI Films.** The effect of the HPH on the WVP of SPI–beeswax films is shown in Figure 1. The WVP of films with a pressure of 0.1 MPa was similar to



**Figure 1.** Effect of the HPH on the WVP of SPI–beeswax films. Data are means  $\pm$  standard deviation ( $n \geq 3$ ). Means with different letters represent a significant difference ( $p < 0.05$ ).

that of the control. The WVP of the films was not influenced by the flow in the homogenizer. The WVP of films with a pressure of 5, 10, and 30 MPa were significantly higher than that of the control. The WVP was statistically similar after the HPH with a pressure of 5, 10, and 30 MPa. In previous studies, the alkali treatment shows no significant influence on the WVP of protein-based films.<sup>13</sup> The enzymatic treatment with transglutaminase reduces the WVP to about 85,<sup>11</sup> 60,<sup>14</sup> and 95%<sup>15</sup> of that of the untreated films (protein-based films). The extrusion reduces the WVP to about 80% of that of the untreated films (protein-based films).<sup>16</sup> The HPH lowered the WVP of SPI–beeswax films to about 50% of that of the control. Therefore, the HPH was effective to lower the WVP of SPI–beeswax films.

The effect of the HPH with a pressure higher than 50 MPa on the WVP of SPI–beeswax films was also evaluated. The WVP of films increased when the pressure of the HPH was higher than 50 MPa. Remarkably, it was difficult to reach the pressure higher than 50 MPa for a HPH treatment in daily

production. Consequently, the results are not presented and are not discussed in the following sections.

**Effect of the HPH on the Microstructure of SPI–Beeswax Films.** The barrier properties of films are related to their microstructure because a loose structure would lead to poor barrier properties.<sup>2</sup> Consequently, the effect of the HPH on the surface and cross-section of SPI–beeswax films is shown in Figure 2. The imaging of the film subjected to the HPH of 0.1 MPa is not present because it is similar to that of the control. The surface and cross-section of the films were compact because of the association of the covalent and noncovalent bonds in the film matrix.<sup>3</sup> Moreover, the surface imaging of SPI–beeswax films became smoother when the pressure of the HPH increased, while the cross-section imaging of SPI–beeswax films was similar. The amount and size of the small particles on the surface of the films after the HPH were smaller than those of the control. The HPH reduced the particle size in the film matrix. Therefore, the reduction of the particle size of SPI–beeswax films was one reason for the reduction of their WVP.

**Effect of the HPH on the Water Adsorption Capacity of SPI–Beeswax Film Solution.** The effect of the HPH on the water adsorption capacity of SPI–beeswax film solution was evaluated by TGA (Figure 3). A similar method had been used to evaluate the water adsorption capacity of some proteins.<sup>23</sup> The TGA curve of the film solution subjected to the HPH of 0.1 MPa is not present because it almost overlaps with that of the control. Specifically, the weight loss of each curve was about 88% because of the moisture loss. Consequently, each solution had the same moisture content of about 12%. However, the equilibrium time when the weight loss reached the 88% level was different. The equilibrium time reduced when the pressure of the HPH increased. The phenomenon reflected that the water adsorption capacity of SPI–beeswax film solution was different. The HPH lowered the water adsorption capacity of the film solution compared to the control. Moreover, the water adsorption capacity of the film solution decreased when the pressure of the HPH increased. These phenomena result from the decrease of the water binding sites in the film matrix.<sup>24,25</sup>

The films subjected to the HPH would have less binding sites for water than the control. Consequently, the WVP of SPI–beeswax films subjected to the HPH would be lower than that of the control, being consistent with the results in Figure 1. Therefore, the reduction of the water adsorption capacity of SPI–beeswax film solution was one reason for the reduction of their WVP.

**Effect of the HPH on the Hydrophobicity of SPI–Beeswax Films.** The contact angle reflects the hydrophobicity of a material.<sup>1</sup> The effect of the HPH on the contact angle of SPI–beeswax films is shown in Figure 4. The contact angle of SPI films is lower than 70° because of their hydrophilic nature.<sup>1,3</sup> The beeswax addition (the control) improved their contact angle to about 75°, and the combination of beeswax addition and the HPH helped to achieve their contact angle of about 125°. Moreover, the contact angle of SPI–beeswax films increased when the pressure of the HPH increased. Hence, the hydrophobicity of SPI–beeswax films increased when they were subjected to the HPH, which showed a similar trend to their WVP and water adsorption capacity (Figures 1 and 3). Similar results have been reported in transglutaminase-treated SPI films<sup>26</sup> and SPI–polylactic acid films.<sup>27</sup> Consequently, the hydrophobicity of SPI–beeswax films was an important factor influencing their WVP. Therefore, the hydrophobic improve-

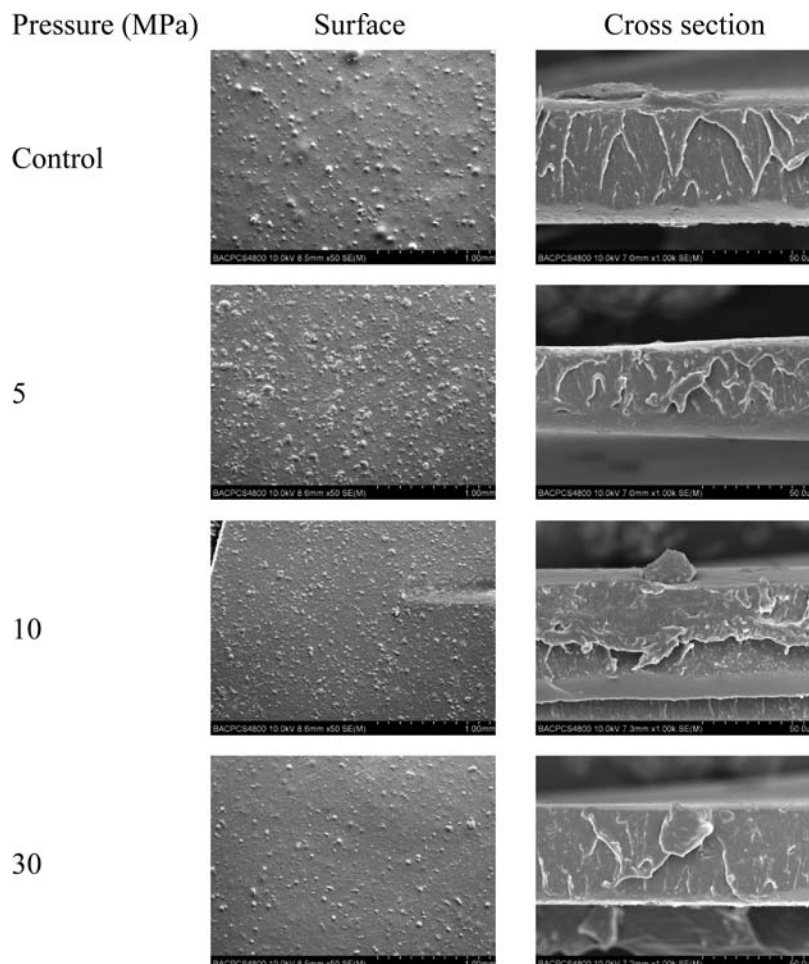


Figure 2. Surface and cross-section profiles of SPI–beeswax films (50 $\times$ ).

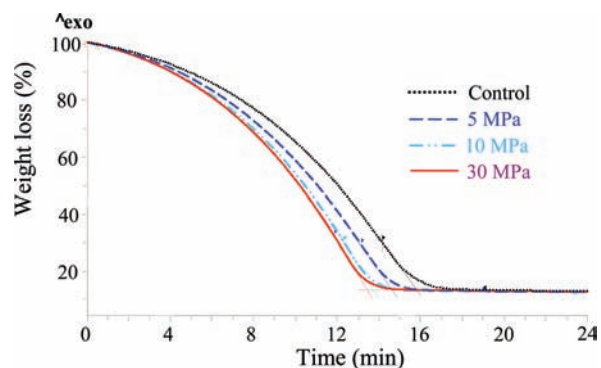


Figure 3. Effect of the HPH on the water adsorption capacity of SPI–beeswax film solution.

ment of SPI–beeswax films was one reason for the reduction of their WVP.

**Effect of the HPH on the Bound-Beeswax Content in SPI–Beeswax Films.** Being the first time, the beeswax content was determined by DSC analysis. At first, the working curve was prepared by beeswax. The beeswax showed an endothermic peak in the DSC profile at 63.7–67.5  $^{\circ}\text{C}$  (Figure 5), with its melting point at about 64.3  $^{\circ}\text{C}$ .<sup>28</sup>  $\Delta Y$  of the DSC profile in 63.7–67.5  $^{\circ}\text{C}$  reflected the melting of the beeswax.  $\Delta Y$  of the beeswax profile was linearly related to its content, with  $R$  of 0.957. Hence, the working curve was  $Y = 0.603X$  [where  $Y$  is  $\Delta Y$  (mW) and  $X$  is the beeswax content (mg)]. In succession, a

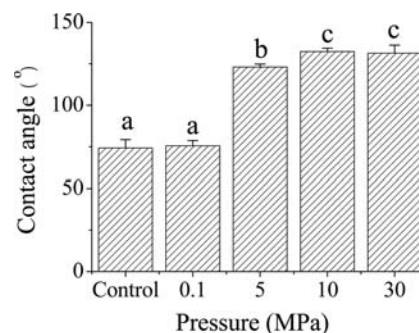
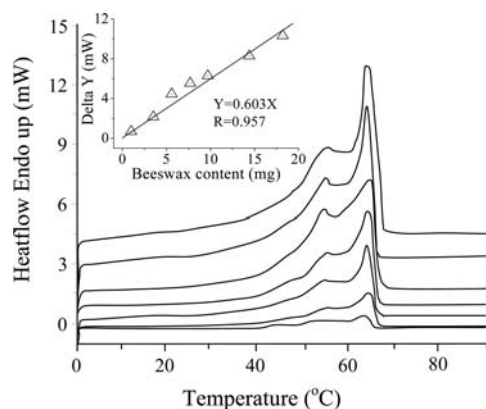


Figure 4. Effect of the HPH on the contact angle of SPI–beeswax films. Data are means  $\pm$  standard deviation ( $n \geq 3$ ). Means with different letters represent a significant difference ( $p < 0.05$ ).

series of trials validated the stability and reproducibility of this method (data not shown). The DSC method showed good stability and reproducibility to measure the beeswax content.

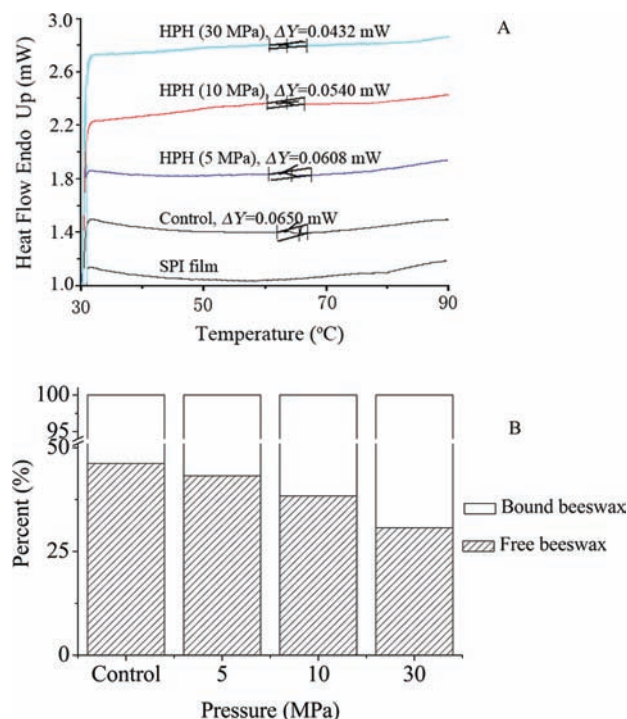
In previous studies, the effects of the beeswax content on the WVP of films have been evaluated.<sup>1,12</sup> However, the effectiveness of the beeswax in SPI–beeswax films was neglected. On the basis of our results, the content and effectiveness of the beeswax influenced the WVP of SPI–beeswax films simultaneously. We classified the beeswax into bound beeswax and free beeswax. The bound beeswax integrates with SPI or glycerol in the film matrix after the HPH, while the free beeswax is kept as small particles in the



**Figure 5.** DSC profiles of the beeswax. The small bar shows the relationship between  $\Delta Y$  and the beeswax content in film solutions.

film matrix. Two hypotheses are given. (1) The free beeswax shows the endothermic peak during heating, while the bound beeswax does not. Consequently, the free-beeswax content could be determined by the DSC analysis. (2) The bound beeswax is related to the hydrophobicity of SPI–beeswax films, while the free beeswax is not. Consequently, the hydrophobicity of SPI–beeswax films is related to the content of the bound beeswax.

The DSC profiles of SPI–beeswax films are shown in Figure 6A. The DSC profile of the film subjected to the HPH of 0.1



**Figure 6.** (A) DSC profiles of SPI film and SPI–beeswax films. (B) Effect of the HPH on the content of free beeswax.

MPa is not present because its  $\Delta Y$  is almost the same to that of the control. The DSC profile of SPI film was smooth and showed no endothermic peak at 63.7–67.5 °C. Thus, the DSC analysis was not disturbed by SPI film, which guaranteed the correctness of the results. The hypotheses were validated. (1)  $\Delta Y$  of SPI–beeswax films subjected to the HPH decreased in comparison to that of the control when their beeswax content

was the same. This phenomenon validated our hypothesis that the bound beeswax would not be detected by the DSC analysis. Consequently,  $\Delta Y$  of the films reflected the content of the free beeswax. (2)  $\Delta Y$  of each film was detected in their DSC profiles.  $\Delta Y$  decreased when the pressure of the HPH increased, which meant that the content of the free beeswax decreased when the pressure of the HPH increased.  $\Delta Y$  showed a similar trend to the WVP and hydrophobicity of SPI–beeswax films (Figures 1 and 3). The phenomenon validated that the bound beeswax was related to the hydrophobicity of SPI–beeswax films.

Figure 6B shows that the free-beeswax content decreased when the pressure of the HPH increased. The phenomenon proved that part of the free beeswax integrated with SPI or glycerol and changed into the bound beeswax in the film matrix when the pressure of the HPH increased.<sup>12</sup> Hence, the HPH increased the content of the bound beeswax, which would lead to a higher hydrophobicity and lower WVP of SPI–beeswax films. Therefore, the bound-beeswax improvement of SPI–beeswax films was one reason for the reduction of their WVP.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

DSC, differential scanning calorimetry; HPH, high-pressure homogenization; SPI, soybean protein isolate; TGA, thermogravimetric analysis; WVP, water vapor permeability

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