

Synergistic effects of sugar beet pectin and laccase on gel properties of egg white

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Abstract: It is of great significance to improve the gel properties of egg white for their related products. This goal was accomplished by investigating the gel properties of egg white via interacting with pectin and laccase, and revealing their mechanisms. Results showed that 0.06% sugar beet pectin (SBP) and 0.5 U/g laccase could endow egg white gels with better texture properties (with the highest hardness of 1 508.00 g, chewiness of 1 144.83, and gumminess of 1 210.57), and superior water holding capacity (WHC) (the highest WHC value of 72.08%). This was attributed to the fact that 0.06% of SBP filled the pores of egg white gel, and enhanced affinity and binding of the gels to water by the good hydrophilicity of SBP, and the decrease in egg white gel pore size. Laccase (0.5 U/g) could promote the cross-linking between the egg white gel and SBP to form a more complex gel network structure through the interactions between the tyrosine residues and ferulic acid in SBP, which brought more ionic bonds (22.47%), hydrogen bonds (12.4%), disulfide bonds (16.97%) and more compact structure to egg white gels. This work can provide an effective method to improve the quality of egg white gel and related products.

Keywords: egg white; gel properties; sugar beet pectin; laccase

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1 Introduction

The oral sensation, flavor release, and sensory enjoyment of food are all directly influenced by the texture qualities of food, which play a significant role in consumption experience. Due to their exceptional ability to gel, egg whites are frequently used for sausages, fish surimi, egg tofu, and other dishes [1–3]. The gelation capabilities of egg white have been extensively studied for its high nutritional value and low potential risk. Egg white gels can produce solid gels as well as serve as water retention agents and thickeners [4–5]. However, single protein gel system has single texture and poor gel mechanical properties. The gelation characteristics of protein-polysaccharide complexes are often superior to those of proteins or polysaccharides alone. Studying the effects of polysaccharides on egg white gels qualities is of great importance for their utilization in preparing gels with a variety of textural and rich flavor characteristics.

Sugar beet pectin (SBP) is a type of natural dietary fiber which is a byproduct of the production of sugar from sugar beets, it is both widely available and fairly priced. Besides, beet pectin is a low-fat pectin, which can form gels in the low sugar concentration, and low-sugar diet is becoming more and more popular [6]. Furthermore, SBP is rich in ferulic acid, which is a phenolic acid with certain antioxidant properties. It can also cross-link with tyrosine residue of protein under catalyst of laccase to form double network hydrogel [7].

As a green and efficient enzyme, laccase has advantage of safety without byproducts. Because of its many active substrates such as various amino acids and plentiful sources, laccase has recently been utilized to research its impact on the textural features of food [8–9]. Besides, it was demonstrated that adding laccase can not only

improve the elastic properties and increase the volume of dough [10], but also promote potato protein polymerization form stronger three-dimensional network structure to enhance thermal stability and anti-shear ability [11]. Covalent cross-linking provided better thermal stability and maintained product hardness and elasticity [12]. Additionally, it was dedicated that the addition of sugar beet pectin and cross-linking agent laccase to soy protein could improve the texture properties of tofu [8]. Therefore, egg white might could be with superior gel properties after interacting with laccase for preparation of better quality gel foods due to its high tyrosine content [13]. However, to the best of our knowledge, the interactions between laccase and egg white have been little studied.

In this study, two kinds of pectin (SBP and high ester pectin, HEP) with different ferulic acid content (2.04% and 1.19%) and laccase (0.5 U/g) were tested to improve egg white gel properties and their mechanisms were tried to be drawn. The physical and chemical properties such as texture, water holding capacity (WHC) and T_2 relaxation time were determined. In a word, this work provides an effective means to improve the gel properties of egg white with pectin and laccase, and proposes their underlying mechanisms.

2 Materials and methods

2.1 Materials

Egg was purchased from Chia Tai Group (Wuhan, China). SBP and a type of HEP were purchased from Guangzhou Hongyuan Food Company (Guangzhou, China) and CPKelco (USA), respectively. Laccase was donated by Anqi Yeast Company. Ferulic

acid were purchased from Shanghai Yuanye Biotechnology Company (Shanghai, China).

2.2 Sample preparation

Fresh eggs were first washed with running water and dried in trays. After that, egg white was separated with an egg separator and collected in a beaker. Then, it was stirred for 10 min with a magnetic mixer and filtered to remove air bubbles and obtain a uniform fresh egg white.

SBP was accurately weighed to prepare 0.4% (*m/V*) solution with deionized water, and the solution was stirred well for 120 min. Then sugar beet pectin solutions of different mass were added to the egg white liquid to form mixtures of egg white containing 5%, 10%, 15%, and 20% (*m/m*) of SBP, and stirred for 1 h until fully dissolved. Laccase with the final enzyme activity of 0.5 U/g was then mixed with egg white and pectin mixture as substrate and the reaction was allowed to stand in a water bath at 40 °C for 2 h. After cooling to room temperature, mixed solution was injected into a 25 mm diameter plastic enteric coating with both ends tied tightly. The inactivation of laccase and formation of egg white gel were by heating in the water bath at 90 °C for 30 min. The activity retention temperature range of laccase is 10–60 °C, and the optimal temperature is 40 °C. Heating at 90 °C for 30 min will denature and permanently deactivate laccase. Finally, it was kept in a refrigerator at 4 °C overnight for further use.

2.3 Determination of ferulic acid content

According to the method reported previously [14], the content of ferulic acid in pectin was evaluated by the characteristic ultraviolet absorption peak at 325 nm. A standard curve was drawn with 20 mg/L ferulic acid standard solution (pH 7.0). PBS was measured as the blank.

2.4 Determination of texture profile analysis

The hardness of the different treated egg white gels was determined using a texture analyzer (TA.XT. plus, Pervicel, USA) with a cylindrical probe (p36R) by compressing the gel (diameter of 25 mm, height of 10 mm) twice, each sample size was cylindrical with a height of 2 cm and a radius of 0.8 cm. The measurement parameters are as follows: 5.0 mm/s pre-test speed, 2.0 mm/s test speed, 5.0 mm/s post-test speed, 50% compression, and a trigger force of 5 g. Texture Loader software was used for data collection and processing.

2.5 Determination of WHC

A certain mass of egg white gel (about 5 g) was weighed and recorded as m_1 . Then it was wrapped with filter paper and put into a 50 mL centrifuge tube. After centrifuging at $429 \times g$ for 10 min at 4 °C, the gel weight was represented by m_2 . The WHC of the gel was calculated using the following equation:

$$\text{WHC (\%)} = \frac{m_2}{m_1} \times 100$$

2.6 Low field nuclear magnetic resonance (LF-NMR)

Egg white gels were cut into squares of uniform size (25 mm \times 25 mm \times 10 mm), placed in cylindrical glass tubes (diameter of 15 mm). The transverse relaxation time (T_2) was measured using the Carre-Purcell-Meiboom-Gill (CPMG) sequence with 0.4 ms echo time, 10 000 echo number and 8 scan number. Finally, the LF-NMR T_2 spectra were obtained by inversion with the MultiExp Inv Analysis software [15].

2.7 Rheological measurements

The rotational rheology of egg whites in the presence or absence of SBP and laccase were determined via a DHR2 rheometer (DHR2, TA Co., USA). First, the temperature increased linearly from 25 to 90 °C at a rate of 5 °C/min, then, kept at 90 °C for 30 min, and decreased from 90 to 25 °C, during which the strain was maintained at 0.3% and the frequency at 1%. To prevent water evaporation, the exposed surfaces of the gels on the parallel plates were covered with liquid paraffin. Then the storage modulus (G') and loss modulus (G'') of each sample were recorded with time and temperature.

2.8 Determination of intermolecular forces

Intermolecular forces were numerically defined as their extractability in solution S_1 , S_2 , S_3 and S_4 caused by breakage of ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds, respectively [16]. Solutions S_1 to S_4 were prepared as: 0.6 mol/L NaCl (S_1); a mixture of 0.6 mol/L NaCl and 1.5 mol/L urea (S_2); a mixture of 0.6 mol/L NaCl and 8 mol/L urea (S_3); a mixture of 0.6 mol/L NaCl, 8 mol/L urea, and 0.5 mol/L β -mercaptoethanol (S_4). Two grams of egg white gel was mixed with each of the above four solutions. Then it was homogenized by a high-speed disperser at 8 000 r/min for 0.5 min and standing at 4 °C for 1 h. The samples were centrifuged at $10\,733 \times g$ for 20 min at 4 °C, and the protein content was measured by biuret method.

2.9 Scanning electron microscopy (SEM)

The freeze-dried egg white gel cut into cubes (5 mm \times 5 mm \times 1 mm) were placed on the sample stage, sprayed with gold, and their microscopic morphology was observed by SEM (JSM-6390LV, NTC Co., Japan) at 500 \times magnification.

2.10 Statistical analysis

All data were expressed as mean \pm standard deviation for three replications. SPSS 26.0 (IBM Corporation, USA) was used for data processing, the differences between groups were analyzed by ANOVA test, and significant differences were considered at $P < 0.05$. Origin 2018 was used for plotting and data processing.

3 Results and discussion

3.1 Content of ferulic acid in different varieties of pectin

It could be seen in Table 1 that SBP had higher content of ferulic acid (2.04%) which was almost as twice as HEP (1.19%). The content of ferulic acid in polysaccharide is closely related to its gel property [17]. Comparing two types of pectin might verify the effect of ferulic acid content on egg white gelation.

Table 1 Ferulic acid content in different types of pectin.

Pectin	$A_{325\text{ nm}}$	Ferulic acid content (%)
HEP	0.112	1.19
SBP	0.237	2.04

3.2 Texture profile of egg white/sugar beet pectin composite gels

Results of the texture profiles were shown in Table 2. Data display that the gel hardness, chewiness, and gumminess of egg whites with the addition of both laccase and pectin were significantly higher

Table 2 Effect of high ester pectin (HEP) and sugar beet pectin (SBP) concentration on the textural properties of egg white gels with laccase (Lac).

Group	Hardness (g)	Springiness	Chewiness	Gumminess
Control	1 423.35 ± 8.34 ^e	0.953 ± 0.002 ^a	1 108.48 ± 3.60 ^f	1 163.30 ± 3.60 ^d
0.01% HEP	1 429.83 ± 9.97 ^{de}	0.952 ± 0.004 ^a	1 124.82 ± 11.47 ^{bc}	1 181.84 ± 12.95 ^{bcd}
0.02% HEP	1 481.34 ± 27.35 ^{cd}	0.939 ± 0.008 ^a	1 131.25 ± 18.09 ^{abc}	1 205.36 ± 17.02 ^{bcd}
0.03% HEP	1 495.64 ± 16.97 ^{bc}	0.937 ± 0.007 ^a	1 121.13 ± 22.29 ^{bc}	1 196.76 ± 16.38 ^{bcd}
0.04% HEP	1 519.14 ± 15.40 ^{bc}	0.947 ± 0.003 ^a	1 159.16 ± 10.14 ^{abc}	1 224.17 ± 12.20 ^{abc}
0.05% HEP	1 494.57 ± 7.70 ^{bc}	0.941 ± 0.003 ^a	1 110.83 ± 15.84 ^{bc}	1 180.41 ± 13.25 ^{cd}
0.01% HEP + 0.5 U/g Lac	1 481.74 ± 2.42 ^{cd}	0.946 ± 0.005 ^a	1 127.79 ± 1.75 ^{bc}	1 192.61 ± 7.49 ^{bcd}
0.02% HEP + 0.5 U/g Lac	1 526.16 ± 26.68 ^{bc}	0.945 ± 0.005 ^a	1 162.81 ± 21.53 ^{ab}	1 230.42 ± 22.86 ^{ab}
0.03% HEP + 0.5 U/g Lac	1 545.61 ± 18.60 ^b	0.943 ± 0.006 ^a	1 159.79 ± 21.02 ^{abc}	1 228.75 ± 14.62 ^{abc}
0.04% HEP + 0.5 U/g Lac	1 603.52 ± 24.36 ^a	0.941 ± 0.009 ^a	1 182.57 ± 18.10 ^a	1 257.29 ± 16.04 ^a
0.05% HEP + 0.5 U/g Lac	1 542.18 ± 13.18 ^b	0.944 ± 0.002 ^a	1 150.56 ± 12.09 ^{abc}	1 218.25 ± 14.86 ^{abc}
Control	1 269.59 ± 13.38 ^e	0.965 ± 0.001 ^a	1 002.15 ± 7.82 ^d	1 037.98 ± 7.60 ^d
0.02% SBP	1 273.12 ± 35.01 ^e	0.951 ± 0.005 ^{bc}	988.18 ± 35.22 ^d	1 038.65 ± 34.41 ^d
0.04% SBP	1 384.00 ± 18.62 ^{cd}	0.958 ± 0.002 ^{ab}	1 080.71 ± 13.30 ^{abc}	1 128.47 ± 15.37 ^{bc}
0.06% SBP	1 364.31 ± 5.50 ^{cd}	0.958 ± 0.004 ^{ab}	1 067.83 ± 6.34 ^{bc}	1 114.02 ± 3.12 ^{bc}
0.08% SBP	1 351.96 ± 27.90 ^{cd}	0.956 ± 0.004 ^{abc}	1 032.48 ± 21.35 ^{cd}	1 079.76 ± 18.94 ^{cd}
0.02% SBP + 0.5 U/g Lac	1 345.65 ± 33.60 ^d	0.954 ± 0.004 ^{abc}	1 034.20 ± 30.88 ^{cd}	1 083.62 ± 32.05 ^{cd}
0.04% SBP + 0.5 U/g Lac	1 464.71 ± 27.08 ^{ab}	0.953 ± 0.002 ^{bc}	1 115.76 ± 25.87 ^{ab}	1 170.93 ± 24.68 ^{ab}
0.06% SBP + 0.5 U/g Lac	1 508.00 ± 12.58 ^a	0.946 ± 0.003 ^c	1 144.83 ± 8.04 ^a	1 210.57 ± 7.39 ^a
0.08% SBP + 0.5 U/g Lac	1 422.43 ± 12.27 ^{bc}	0.949 ± 0.004 ^{bc}	1 085.09 ± 7.45 ^{abc}	1 143.28 ± 4.06 ^{bc}

Note: the data in the same column marked with different lowercase letters are significantly different ($P < 0.05$). SBP, sugar beet pectin; HEP, high ester pectin; Lac, laccase.

than those of egg whites with the addition of pectin only or the control. As what it shows, HEP could significantly enhance hardness from 1 423.35 to 1 519.14 g, chewiness from 1 108.48 to 1 159.16, and gumminess from 1 163.30 to 1 224.17 (0.04% HEP, m/V) on the basis of the control ($P < 0.05$). Furthermore, the addition of 0.5 U/g (egg white liquid) of laccase could further improve the texture properties of the gels based on the addition of 0.04% of HEP. The value achieved to 1 603.52 g (hardness), 1 182.57 (chewiness), and 1 257.29 (gumminess). Similarly, the addition of 0.06% (m/V) of SBP and 0.5 U/g laccase could significantly further improve the texture properties of egg white on the basis of 0.06% (m/V) of SBP ($P < 0.05$).

3.3 WHC of egg white/sugar beet pectin composite gels

As shown in Figure 1(A), although the single SBP (0.02%–0.08%, m/V) had not significantly improved the WHC of egg white gels ($P > 0.05$), the addition of 0.06% of SBP and 0.5 U/g of laccase increased the gels' WHC significantly from 65.08% (the control) to 72.08% ($P < 0.05$). Numerically speaking, 0.06% SBP and 0.5 U/g laccase increased 4.14% of the gels WHC based on the single SBP (0.06%) and 7.00% on the natural egg white gels.

3.4 LF-NMR of egg white/sugar beet pectin complex gels

The water distribution of the gels was shown in Figure 1(B) by transverse relaxation time (T_2) of LF-NMR. As reported, the peaks of T_{21} (1–10 ms), T_{22} (50–300 ms), and T_{23} (500–2 000 ms) were related to bound water, immobilized water and free water, respectively [18]. Result showed that with the increase of SBP, the immobilized water of the gels decreased obviously from 100% (the

control) to 97.48% (0.06% SBP). The bound water of the gels was increased significantly from 2.52% (0.06% SBP) to 8.98% after the addition of 0.5 U/g of laccase, and the immobilized water decreased 6.46%.

3.5 Rheological properties

Figures 2(A) and 2(B) showed the changes of G' and G'' of egg white gels with only 0.06% SBP and egg white gels with 0.06% SBP and 0.5 U/g laccase, respectively. All the egg white/sugar beet pectin composite solution increased from 25 to 90 °C at a rate of 5 °C/min, and kept at 90 °C for 30 min and then cooled down to 25 °C at a rate of 5 °C/min. It could be seen that the G' of samples was higher than the G'' . The G' and G'' shared the similar trend of both positive correlation with the increase of temperature. Besides, the loss tangent values were less than 1. It was showed that sudden jump of the G' and G'' occurred at about 50 °C, indicating that the temperature at which the gel is formed from this mixed solution might be 50 °C. Comparing Figures 2(A) and 2(B), it could be found that the G' and G'' of the composite gels had a slight decrease after the addition of laccase. During the cooling phase, the G' and G'' both increased obviously.

3.6 Intermolecular forces

Figure 3 showed that the overall proportion of intermolecular forces in each gel sample from strong to weak were ionic bonds, disulfide bonds, hydrophobic interactions and hydrogen bonds. On the whole, ionic bonds (20% of the control, 20.2% of 0.5 U/g laccase) and hydrogen bonds (10% of the control, 10.73% of 0.5 U/g

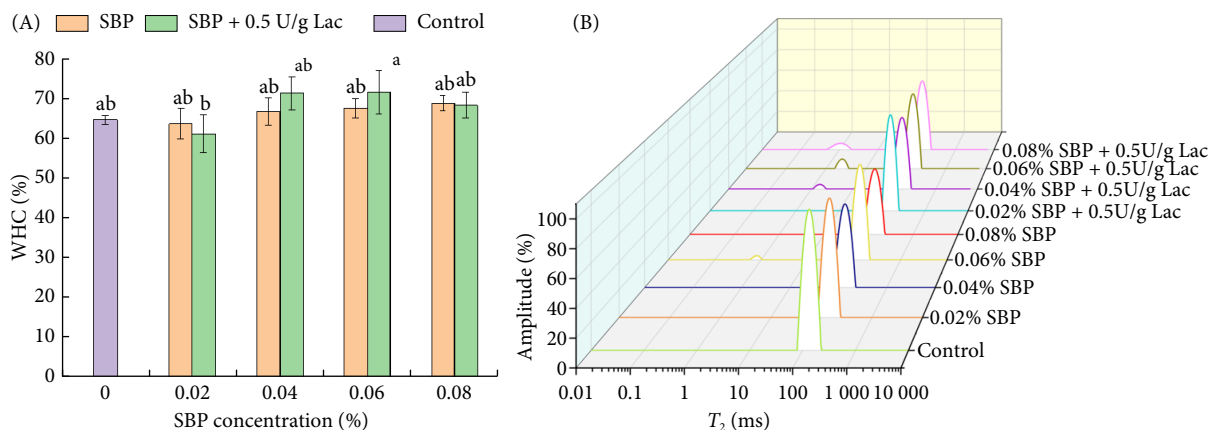


Figure 1 (A) WHC of egg white/SBP composite gels and (B) T₂ relaxation time analysis of egg white/SBP composite gels. Different letters indicate significant differences ($P < 0.05$). SBP, sugar beet pectin; Lac, laccase.

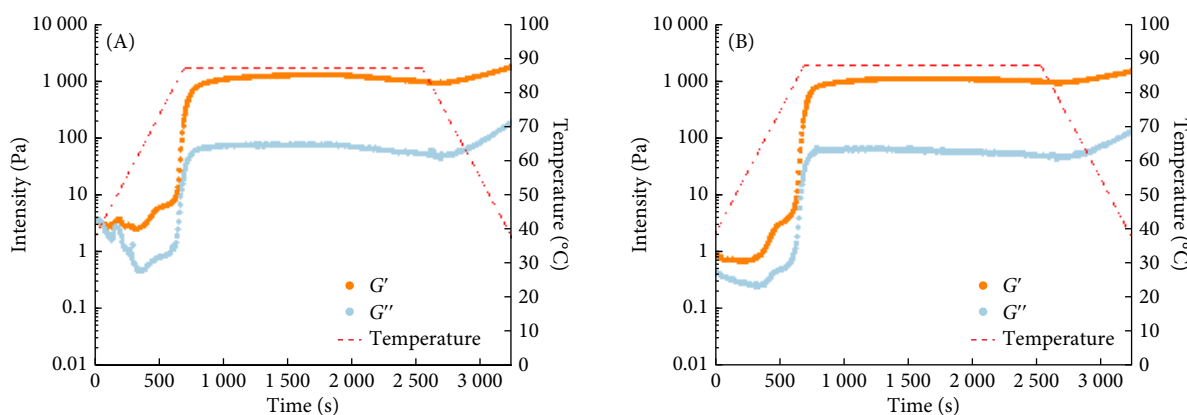


Figure 2 Rheological changes of egg white/suger beet pectin composite gels. (A) Egg white solution with 0.06% suger beet pectin; (B) Egg white solution with 0.5 U/g laccase and 0.06% suger beet pectin.

laccase) increased, while the hydrophobic interactions (13.47% of the control, 14.03% of 0.5 U/g laccase) decreased significantly after the addition of 0.06% SBP and 0.5 U/g laccase ($P < 0.05$). There ionic bonds, disulfide bonds, and hydrogen bonds increased by 2.47%, 0.27%, and 2.4% compared to control, respectively, after the addition of laccase (0.5 U/g) in the egg white complex with 0.06% SBP. However, the hydrophobic interactions decreased 2.13% with laccase than adding 0.06% SBP.

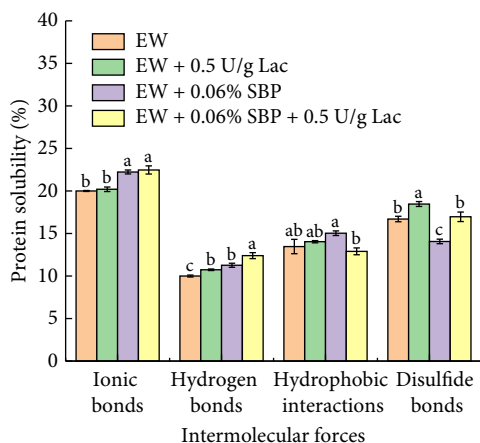


Figure 3 Intermolecular forces in egg white/beet pectin complex gels. Different letters indicate significant differences ($P < 0.05$). EW, egg white; SBP, suger beet pectin; Lac, laccase.

3.7 Micromorphology

Figure 4 showed the micromorphology of egg white gels by SEM at a magnification of 500 ×. It could be seen that the surface of the egg white gels without the addition of pectin was uneven and had obvious holes. After the addition of SBP, the holes reduced and the surface of the egg white gels was more flat, uniform and dense. The most regular microstructure of the egg white gels surface was observed with the addition of 0.06% SBP and 0.5 U/g laccase, which showed a significant reduction in pore size and the pores became more dense and homogeneous.

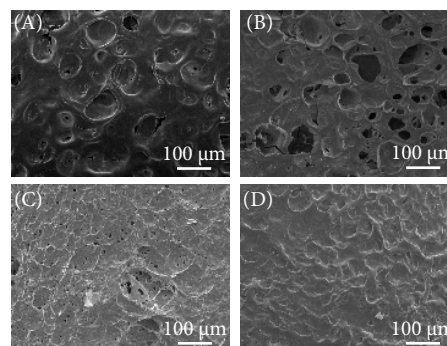


Figure 4 Micromorphological analysis of egg white/suger beet pectin composite gels (× 200). (A) Egg white; (B) Egg white + 0.5 U/g laccase; (C) Egg white + 0.06% SBP; (D) Egg white + 0.06% SBP + 0.5 U/g laccase.

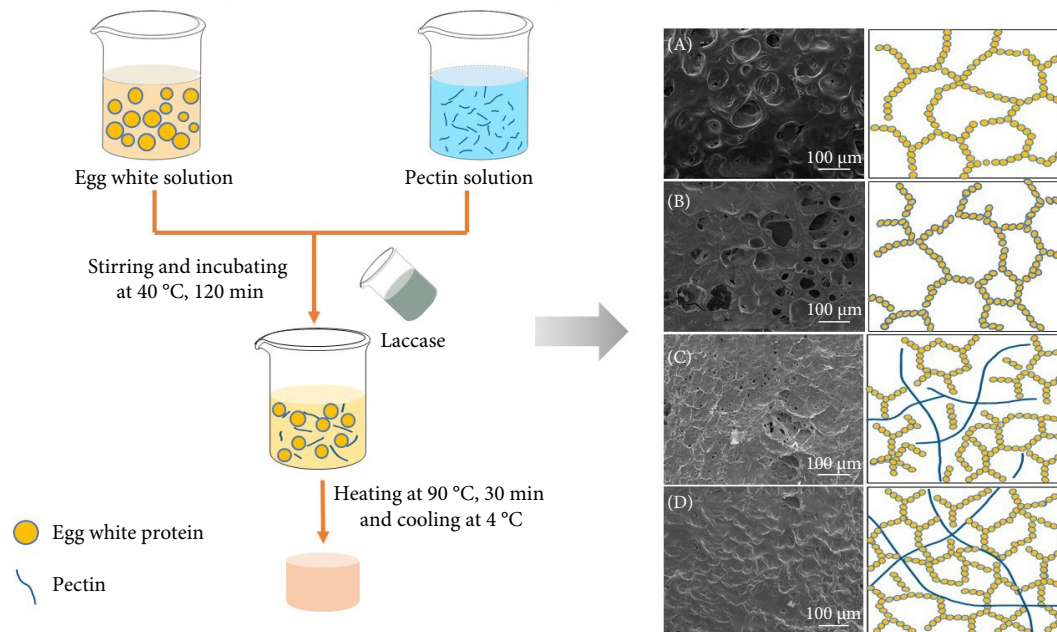


Figure 5 The underlying mechanisms proposed for promotion of 0.5 U/g laccase and 0.06% sugar beet pectin on the gel properties of egg white. (A) Egg white; (B) Egg white + 0.5 U/g laccase; (C) Egg white + 0.06% SBP; (D) Egg white + 0.06% SBP + 0.5 U/g laccase. The magnification is 200.

3.8 Propos of the underlying mechanism

The underlying mechanisms of pectin and laccase improving the gels properties of egg white were proposed as Figure 5. The natural egg white gel was with loose and porous network structure. 0.5 U/g laccase could improve cross-linking of the protein chains and partially increase compactness of the gel network, but there were still pores of different sizes. 0.06% SBP could fill the pores of egg white gel matrix, reduce the large pores of the gel, thus increasing the gel strength and WHC of egg white gels. Furthermore, 0.5 U/g laccase could further enhance the the gel properties based on the addition of 0.06% SBP by increasing the cross-linking between ferulic acid in pectin and tyrosine residues in protein.

4 Discussion

Ferulic acid as a natural phenolic acid has excellent capacity of oxidation resistance [19]. What's more, ferulic acid can be treated as an excellent bridging mediator to enhance the network structure of protein gels. Therefore, the ferulic acid content of pectin was measured to find out the intrinsic reason why pectin promoted the gelatinization of egg white. It was reported that under the promotion of laccase, ferulic acid could cross-link with tyrosine in protein to improve the gel quality [8]. In addition, laccase had a significant impact on the microstructure of whey protein and sugar beet pectin polymer, which could promote its formation of a more refined network structure [20]. Therefore, pectin and laccase were selected as effective means to enhance the gel properties of egg white, and their underlying mechanism was attempted to be revealed in this work.

Polysaccharides as natural gelling agents have significant effects on egg white textural properties. Textural property is not a single property but a collection of physical properties influencing the structure of the food [21]. And the gel hardness, springiness, chewiness and gumminess in TPA reflect the textural changes of egg whites from different perspectives. After the addition of laccase, egg white gel had a higher hardness, chewiness and gumminess,

which might be due to egg white gel crosslinking with sugar beet pectin by ferulic acid. Furthermore, because ferulic acid is an excellent reaction substrate for laccase, sugar beet pectin with higher content of ferulic acid had better improvement. A suitable pectin concentration helped to improve the ability that gels resist external forces and improve taste. Laccase can promote the cross-linking of egg white and sugar beet pectin by cross-linking ferulic acid in sugar beet pectin and tyrosine in egg white proteins [7], which improved the interactions between egg white and sugar beet pectin.

The WHC represents product's ability to maintain water from resisting external influences, since water could be easily lost during cooking and processing [22]. A better WHC can provide a rich flavor and juiciness of the food. The WHC of the composite gel had an increase after the addition of SBP, which might be attributed to it that the intermolecular interaction between egg white and polysaccharide increased the non-covalent bonds such as hydrogen bonding, which improved the ability of egg white gel to absorb water. The interaction between egg white protein and SBP provides sufficient WHC for egg whites, which formed a denser network gel under the action of laccase and could trap more water.

It was showed that the microstructure of the gel is also closely related to the WHC of egg whites, with uniform, dense microstructures of small pore networks having better WHC than those with loose, large pores [23]. By observing the SEM images, it was found that the pure egg white gel surface had the largest pores and easily dissipated water. In addition, after adding 0.06% sugar beet pectin to the egg white, the gel surface microstructure became denser and the pore size became smaller. While the egg white gel with the addition of both beet pectin and laccase had reduced porosity and the most uniform and dense microstructure, therefore, their WHC was the highest. These conclusions were similar to the research of Zhuang et al. [24]. In addition, the moderate amount of polysaccharides as hydrocolloids could improve the WHC of protein which might be due to the formation of a denser gel network in the egg white gel after the addition of laccase [25]. Besides, the cross-linking of tyrosine in egg white proteins and

ferulic acid in beet pectin formed a dense network under the action of laccase, thus it could bind more water.

LF-NMR technique has been successfully applied to reveal water distribution in many food systems which is also an effective method to evaluate the water retention of gels [26–28]. During NMR, the hydrogen atoms in food and water are to revolve around the nucleus with an electric charge before continuing to rotate on their own. This is similar to how a magnet works and how the food tissue is made up of many hydrogen nucleus magnets as a whole [22]. The longer the T_2 time, the more relaxed the water binding was to the sample and the easier to be lost [29]. The increase of tightly bound water content in the gel with the concentration of pectin, which might be due to the fact that pectin was a hydrophilic colloid with better water retention and could retain more water [30–31]. Besides, the bound water content of the egg white/beet pectin composite gels increased significantly after the addition of laccase, which probably be due to the dense gel network structure resulting from the further cross-linking of egg white and beet pectin after the addition of laccase, thus trapping more bound water. Moreover, the equation of laccase catalyzed reaction between protein and beet pectin [32] showed that laccase catalyzed oxidation was environmentally friendly.

The rheology could reflect the temperature and viscoelasticity of gel formation in food products. The G' of all samples was higher than the G'' , which proved that the samples were able to form gels. In addition, studying the changes of rheological properties about egg white after the addition of laccase and beet pectin could help the processing and production of gel products. The indicators of rheology such as G' and G'' reflect the changes of rheological properties of the samples. As shown in the results, the loss tangent values were less than 1, which indicated that the gel samples were more elastic than viscous and form elastic gels. The sudden jump of G' and G'' in the rheological addition of the composite gel sample occurred at about 50 °C, indicating that the temperature at which the gel is formed from this mixed solution might be 50 °C. After the addition of laccase, the viscoelasticity of gels decreased, which might be due to intermolecular aggregation. During the cooling phase, the G' and G'' increased significantly, which might be due to the rearrangement of the network structure during the cooling phase and the lower entropy drive. This phenomenon improved the development of non-covalent interactions between the aggregated molecules in the egg white gel network [33].

Intermolecular forces are the interaction forces between protein molecules which play a role in maintaining the structural stability of the gel network. Besides, intermolecular forces are mainly composed of non-covalent (ionic bond, hydrogen bond, hydrophobic interaction) and covalent (disulfide bond) bonds [34]. The appropriate ratio of the composition of these intermolecular forces was the basis for excellent protein gel properties [16]. By breaking ionic bonds, hydrogen bonds, hydrophobic interactions, and disulfide bonds by chemical reagents and measuring the solubility of proteins in the corresponding chemical solvents, the ratios of these four intermolecular forces could be derived and used to analyze the mechanism of composite gels.

The disulfide bonds are stable covalent bonds between protein molecules. The increase in disulfide bonds after the addition of laccase might be due to laccase's promotion of the cross-linking between egg white protein and beet pectin, where more sulfhydryl groups were exposed and interconverted with disulfide bonds [35]. Hydrogen bonds as strong non-covalent bonds, maintain protein

secondary structure. From the equation about laccase reaction [32], it could be seen that the product of catalytic reaction was water which contains many hydrogen bonds. The increase in hydrogen bonds indicated the enhancement on affinity of the composite gel with water, which was partly responsible for the increase of WHC of the gels. In contrast, the hydrophobic interactions relied mainly on the exposure of hydrophobic groups. In a word, the addition of 0.5 U/g laccase and 0.06% SBP contributed to increasing formation of hydrophilic bonds or interactions, thus increasing the WHC and gel properties.

5 Conclusion

This work aims to improve the gel properties of egg white by interacting with pectin and laccase. Results showed that 0.06% SBP and 0.5 U/g laccase could endow egg white gels with better texture properties (with the highest hardness of 1 508.00 g, chewiness of 1 144.83, and gumminess of 1 210.57), and superior WHC (holding the highest WHC value of 72.08%). Their mechanisms were proposed as: i) 0.06% of SBP could be filled in the pores of egg white protein gel network, which enhanced the affinity and binding of egg white gel to water by the good hydrophilicity of SBP and the reduction of in egg white gel pore size; ii) Laccase (0.5 U/g) could promote the cross-linking between the egg white gel and SBP to form a more complex gel network structure through the interactions between tyrosine residues and ferulic acid in SBP. In conclusion, SBP and laccase improved egg white gel properties, not only to enhancing the gel properties of egg white by more interactions, but also to bringing more compact and complex network structure or better gel strength to the gels. This work can provide an effective method to improve the quality of egg white gel and related products.

Conflict of interest

The authors have no conflict of interest to declare.

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