

Coating Effect of Whey Protein and Xylose Maillard Reaction Products on Walnut Lipid Peroxidation

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Abstract—**BACKGROUND:** Whey Protein Isolate (WPI) film is good barrier to oxygen but poor barrier to water vapor. Maillard Reaction (MR) between protein and sugar could modify protein structure. Our study used xylose (Xyl) to modify WPI through MR, and prepared WPI-Xyl MR product (MRPs) film. Its mechanical property, water vapor permeability, especially coating effect on lipid peroxidation of walnut coated were assessed. **RESULTS:** Mechanical properties of WPI-Xyl MRPs film have been improved, with the penetration and tensile strengths of 1.7 times and 2.4 times that of WPI film, respectively; while water vapor permeability was 24% lower for WPI-Xyl MRPs film than WPI film. The acid value and TBARS value were lower, 21% and 16%, respectively, for the walnuts coated with WPI-Xyl MRPs film than un-coated. While the walnut coated with WPI film had no effect on lipid peroxidation development. **CONCLUSION:** WPI-Xyl MRPs film has good mechanical property and low water vapor permeability. The coating with WPI-Xyl MRPs film, but not WPI film, could effectively delay lipid peroxidation of walnut. MR could be used as an effective method to improve film property and coating effect of WPI.

Index Terms—whey protein, Maillard Reaction Products (MRPs), coatings, walnut, rancidity

I. INTRODUCTION

In recent years, an increased interests on biodegradable films and coatings have been generated by consumer demands for high-quality food product and environmental awareness of plastic packaging materials. Edible coatings could enhance food quality by acting as protecting layer for food product along with the primary package [1]. They could also be used as effective carriers for functional ingredients, such as antioxidants, antimicrobial agents, flavorings, and pigments [2]. Materials that are commonly used for edible coatings include proteins, such as wheat gluten, corn zein, and casein; polysaccharides, such as starch, pectin, and alginate; and lipids, such as glycerol esters, waxes, and resin. While proteins have been considered as the most significant edible packaging materials, because protein films generally have good mechanical and optical

properties and are good barriers against oxygen permeation [3].

Whey Protein Isolate (WPI) film was an excellent oxygen barrier at low or intermediate RH, but with low tensile strength and high water vapor permeability due to its hydrophilic nature. It has been shown that heating could modify protein from the native globular structure to unfolded protein strands, exposing internal SH and hydrophobic groups, which led to intermolecular S-S bonding and hydrophobic interactions upon drying to form films [3], [4]. The fragility of the film makes the addition of plasticizers necessary, which not only provides flexibility to the film but also increases its water vapor permeability (WVP) [2], [5]. Some small molecules have been added to improve the mechanical or barrier property of WPI film, such as cystine, cinnamaldehyde, and ferulic acid [4], [6].

Edible coating was used for nuts to reduce the oxygen exposure and delay oxidative rancidity [7]. Walnut has high lipid content of approximately 65%, of which about 70% was polyunsaturated fatty acids (PUFA). The major fatty acid was linoleic acid (C18:2 ω 6), followed by oleic acid (C18:1 ω 9), α -linolenic acid (C18:3 ω 3), palmitic acid (C16:0), and stearic acid (C18:0) [8], [9]. Such high content of fatty acid renders walnut readily to lipid peroxidation during storage, which leads to the development of undesirable off-flavors resulting from lipid peroxidation and reduces the acceptance and quality of walnut products [7], [8].

Lipid peroxidation is affected by many factors, such as temperature, oxygen concentration, relative humidity (RH), and light. It has been reported that hexanal content (oxidative products) and peroxidative value were higher for the walnuts stored at 21% oxygen atmosphere and 53% RH than at less than 2.5% oxygen and 21% RH [7]. Walnut preserved good quality when packaged by high barrier material and stored at low temperature [10]. The similar result was also observed, using oxygen absorber in packaging system [11]. These reports demonstrated the possibility of using coating materials with good barrier property to water vapor and oxygen to delay the walnut lipid peroxidation.

WPI coating has been used as protective barrier to reduce oxygen uptake and rancidity in roasted peanut [3]. While it was reported that development of lipid

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peroxidation in the WPI coated walnut was not different from that of untreated walnut [8]. It has been shown that conjugation between whey protein and dextran significantly increased antioxidant activity of whey protein. Furthermore, the whey protein–dextran conjugate could also significantly increase the ABTS radical scavenging activity of heated bog bilberry anthocyanin extract [12]. Our preliminary experiment revealed that when WPI was conjugated with xylose (Xyl) its gelling property could be improved (data not shown).

The present study assessed the film forming characteristics of WPI and WPI-Xyl MRP, along with physiochemical and mechanical properties, and water vapor permeability of both WPI and WPI-Xyl MRPs films. The coating effects of WPI film and WPI-Xyl MRPs film on development of the lipid oxidation of walnut were further investigated.

II. MATERIALS AND METHODS

A. Materials

Walnuts were purchased from the local market. Whey Protein Isolate (WPI) with a minimum protein content of 95% was obtained from Davisco Foods International (MN, USA). Xyl was provided by Jia Kangyuan Co.Ltd (Beijing, China). Glycerol (Gly) was purchased from Jingshun Chemical Reagents Co. Ltd (Beijing, China). Ethanol, ether, and KOH were all provided by Beijing Chemical Reagents Co. Ltd (Beijing, China). Trichloroacetic acid and thiobarbituric acid were obtained from Tianjin Kemio Chemical Reagents Co. Ltd (Tianjin, China) and Sinapharm Chemical Reagents Co. Ltd (Beijing, China), respectively.

B. Preparation of Film Solutions

20 mL Gly was mixed with 20 mL dH₂O as the stock solution of 50% Gly. The stock solution of WPI or Xyl was prepared by dissolving WPI or Xyl in deionized water (dH₂O) to a final concentration of 20% (g/mL), and they were mixed in preset ratio to obtain film-forming solutions. The concentrations for WPI were adjusted to 9-11% (g/mL) and Gly to 9-11% (mL/mL) for WPI film-forming solutions. The concentration of WPI was adjusted to 10% (g/mL), Xyl to 2.5-7.5 % (g/mL), Gly to 4-6 % (mL/mL) for WPI-Xyl MRPs and their film-forming preparation.

Heating temperatures and time for the WPI and WPI-Xyl MRPs film-forming were chosen based on their optimum physical and mechanical properties, and water permeability. The WPI solution was maintained at 80°C for 30 min, and the WPI-Xyl MRPs solution was heated at 80°C for 45 min or 90°C for 30 min. The heated solutions were cooled down to room temperature (20°C) and then added Gly into the solutions. 5.5 mL of WPI solution and 6.5 mL of WPI-Xyl MRPs were put in the dishes, respectively, and left the solutions at room atmosphere to form films.

C. Physiochemical and Mechanical Property Assessment

The thickness of film was measured using micrometer calipers, and expressed as an average of eight readings

taken randomly at 1 cm from the film edge. The absorbance of film at 550 nm was also recorded to show the transparency of film [13]. The mechanical properties of the film were measured by texture analyzer (TMS-PRO, Food Technology Corporation). To measure the tensile property, rectangle film (6 cm × 2 cm) was prolonged at a rate of 60 mm/min. The largest tensile strength was recorded to reflect the hardness of film: $T_s = F_t / (L * W)$, where F_t is the max puncture strength (N); L is the thickness of the films (mm); W is the width of the films (mm) [14]. While the elongation percentage ($E\%$) that related to the elasticity of film was calculated according to the following formula: $E(\%) = \Delta L / L_0$, of which ΔL was the change of the film length during the elongation, and L_0 was the initial length of film. To measure the puncture strength, the film with the shape of half circle was placed in the middle of two polymethacrylate plates with a whole whose diameter was 3.2 cm, and was penetrated by a cylindrical probe (2 mm in diameter) at the rate of 60 mm/min. Puncture strength (PS, N/mm) and puncture deformation (PD, mm) were calculated from the stress-strain curves: $P_s = F_p / L$, where F_p is the max puncture strength (N); L is the thickness of the films (mm); PD is the fall distance of the probe when the films were punctured [15].

D. Water Vapor Permeability Assessment

Water vapor permeability (WVP) was determined using the cup method. The polymethyl methacrylate cups with the area of 0.785 cm² were used for the experiment. Anhydrous calcium chloride was put into the cup to provide relative humidity (RH) of 0%, and sealed the film onto the O ring of the cup. The cups were then placed in a desiccator containing saturated KBr with RH 80% at room temperature (20 °C) [16]. The weight of cup was recorded every 40 min during the 4 h of period time. The value of WVP was calculated according to a formula: $WVP = \Delta m * d / A * T / \Delta P$. Δm is the weight change of cups within certain time (g), d is the thickness of film (mm), A is the area of cup (m²), T is the time interval (h), and ΔP is the difference of water vapor pressure inside and outside of the cup (kPa) [17].

E. SDS-PAGE

5% stacking gel and 15% separating gel were used. The WPI solution (50 µL) was diluted by dH₂O to the final protein concentration of 1 mg/mL, and was mixed with the treatment solution (50 µL) and maintained in the boiling water for 5 min. 17.5 µL of the treated sample solution was loaded on the gel set. Gels were put in the Tris-HCl buffer (pH 8.3). Electrophoresis was run at 80 V for 30 min, and then changed into 120 V for 2 hours. The gel was stained overnight with one-step Coomassie Blue stain [0.25% Coomassie Brilliant Blue R-250 in methanol/water/glacial acetic acid (50/40/10, v/v/v)], and destained in a solution of methanol/water/glacial acetic acid (30/60/10, v/v/v) [18].

F. Walnut Coating and Accelerated Lipid Peroxidation Process

Walnut kernels with similar size (2.5-3.0 cm in diameter) were carefully chosen. 150 g of the walnut

were immersed in the 200 mL film forming solutions for 2 min, with gently stirring to make sure that all pieces were evenly coated. The coated walnuts were lift with slotted spoon onto parchment paper and dry at room temperature for 12 h before further experiments. Walnut were then divided into five groups: “control (unheated)” referred to walnuts that were stored at room condition (28°C) without coating and heating treatment; walnuts in the “control (heated)” group were stored at 50°C, RH 80% without coating; “WPI” is for the walnut coated by WPI film [10 % (g/mL) WPI, 10% (mL/mL) Gly]; the walnuts in “MRPS -80” and “MRPs-90” were all coated by WPI-Xyl MRPs films [10% (g/mL) WPI, 5% (g/mL) Xyl, 5% (mL/mL) Gly, but were prepared at 80 °C for 45 min or 90 °C for 30 min, respectively. All film coating walnut groups were stored at 50°C, RH 80% for accelerated lipid peroxidation test.

A desiccator contained saturated KBr with about RH 80% at 50°C (in oven) [16] was used for walnut storage accelerating experiment. 30 g of walnut were collected every 5 days and milled to paste in a mortar. The paste was soaked in 150 mL petroleum ether for 12 h. The blend was transferred into 50 mL tube carefully using teaspoon, and centrifuged at 2800×g for 10 min. The supernatant was collected and evaporated at 40°C to obtain the oil for lipid peroxidation analysis.

G. Acid Value Measurements

Acid value refers to the weight of KOH (mg) needs to neutralize 1 g of lipid. 2.2 g of lipid was added into 50 mL solutions containing ethanol and ether (1:1, v/v). Use 0.1 mol/L KOH to neutralize the lipid and record the volume consumed. Phenolphthalein was used as indicator. Acid value was calculated by following formula:

$$\text{Acid value} = C_{\text{KOH}} * V_{\text{KOH}} * 56.11 / M_{\text{lipid}}$$

C_{KOH} was the mole concentration of KOH (mol/L), V_{KOH} is the volume of KOH consumed (mL), and M_{lipid} was the weight of lipid (g) [19].

H. TBARS Measurements

Thiobarbituric acid reactive substances (TBARS) were measured by combining 0.5 mL of oil with 0.5 mL of water and 2.0 mL of TBA reagent (prepared by mixing 15 g trichloroacetic acid, 0.375 g thiobarbituric acid, 1.76 mL of 12 N HCl and 82.9 mL of dH₂O) in test tubes and placed in a boiling water bath for 10 min. The tubes were cooled to room temperature and then centrifuged (1000×g) for 10 min. The absorbance (Abs) of the supernatant was measured at 520 nm [20].

I. Statistical Analysis

All experiments were performed in triplicate. The values are presented as mean ± SD. The means were compared by one-way ANOVA, followed by Tukey's comparisons, using the M SAS 9.1 TS Level 1M3 for Windows (SAS Institute Inc., Cary, NC, USA). The level

of confidence required for significance was set at $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Physical and Mechanical Properties of WPI Film

Physical and mechanical properties of WPI film with different concentrations of both WPI and Gly were shown in Table I. Thickness of film increased from 0.13 mm to 0.20 mm with the increasing of WPI-Gly concentration. Absorbance of the WPI film was about 0.1 and did not change with the WPI-Gly concentration. Puncture strength decreased with the increasing of WPI-Gly concentration. While there was no significant difference for the three groups in the puncture deformation, the elongation strength, and elongation percent.

Increasing concentration of WPI from 7% (g/mL) to 10% (g/mL), along with constant Gly concentration, could help to obtain thicker film [20]. Ref. [6] reported that film made from 9% (g/mL) WPI was thicker than that made from 5% (g/mL) WPI at equal WPI:Gly ratio. The high density of WPI solution was associated with film thickness.

The WVP of WPI film increased from 0.19 g mm/kPa h m² to 0.39 g mm/kPa h m² as WPI concentration increased from 9% to 11% for WPI film (Fig. 1), which indicated the decrease of water barrier property. Similar results were reported by Ref [6]. It might be explained by the formation of larger pores and increase of liquid phase (water and plasticizer) at high concentrations. It is easy for water to go through the film with more hydrophilic liquid phase. Based on comprehensive comparison, 10% WPI was chosen as an optimum concentration in further film-forming experiments.

Physical and mechanical properties of WPI film with change of only Gly concentrations were also shown in Table I. The thickness of the film was increased as Gly concentration increased. As Gly concentration increased from 5% to 15%, puncture strength was decreased from 36.4 N/mm to 3.5 N/mm while the puncture deformation was increased from 1.94 mm to 3.13 mm at the same time. Similarly, the tensile strength was decreased from 9.57 N/mm² to 0.26 N/mm², and the elongation percent was increased from 59% to 81%.

Gly is polar, water-soluble, with a high boiling point, which make it a good plasticizer for protein film [6]. The -OH groups of Gly could interact with -NH₂, -NH-, -COOH, and decrease the inter- and intra-molecular interactions such as hydrogen bond and improve the motion ability of protein macromolecules. Increase of Glc was related to the decrease of puncture and tensile strength of WPI film, as well as the increase of puncture deformation and elongation percent.

WVP of WPI film was increased from 0.24 to 0.43 g mm/kPa h m² as Gly was increased from 5% to 15% in the films (Fig. 1), which was associated with the decrease of water barrier property.

TABLE I. PHYSICAL AND MECHANICAL PROPERTIES OF WPI FILMS PREPARED WITH DIFFERENT CONCENTRATIONS OF WPI AND GLY

WPI:Gly (w:v, %)	Physical property		Mechanical property			
	Thickness (mm)	Abs (550nm)	PS (N/mm)	PD (mm)	TS (N/mm ²)	E% (%)
Changes of both WPI & Gly conc.:						
9 : 9	0.13±0.01 ^a	0.095±0.010 ^a	13.8±0.6 ^a	4.00±0.12 ^a	0.95±0.10 ^a	80±6 ^a
10 : 10	0.17±0.01 ^b	0.095±0.005 ^a	11.3±0.4 ^b	4.13±0.06 ^a	0.84±0.08 ^a	66±10 ^a
11 : 11	0.20±0.01 ^c	0.103±0.005 ^a	9.8±0.6 ^c	4.16±0.21 ^a	0.84±0.03 ^a	63±6 ^a
Change of only Gly conc.:						
10 : 5	0.08±0.01 ^a	0.098±0.011 ^a	36.4±3.3 ^a	1.94±0.28 ^a	9.57±0.19 ^a	59±2 ^a
10 : 10	0.17±0.02 ^b	0.102±0.010 ^a	7.7±0.6 ^b	2.75±0.02 ^{ab}	1.04±0.19 ^b	66±5 ^a
10 : 15	0.22±0.01 ^c	0.136±0.027 ^a	3.5±0.8 ^c	3.13±0.54 ^b	0.26±0.01 ^c	81±3 ^b

PS, puncture strength; PD: puncture deformation TS: tensile strength; E: elongation at break. Data were presented by mean \pm SD (n=3), and the means in the same column with different letters had significant difference (p<0.05)

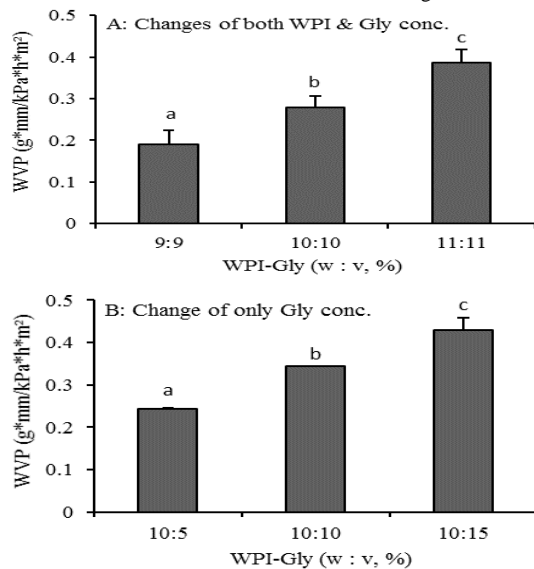


Figure 1. WVP of WPI films prepared with different concentrations of WPI and Gly

WPI:Gly: the mixtures of WPI and Gly were prepared (A) at the concentrations of 9%, 10%, and 11% for both WPI (g/mL) and Gly (mL/mL); (B) at concentrations of 10% (g/mL) for WPI, 5%, 10%, and 15% (mL/mL) for Gly, respectively. The column values with different letters have significant differences (p<0.05, n=3).

B. Development of WPI and Xyl Maillard Reaction (MR)

Absorbance change of heated WPI and Xyl system was shown in TABLE II. Both 80 °C and 90 °C were chosen since they were commonly applied in WPI film forming studies. The absorbance at 450 nm were increased by 0.032 and 0.085 after being heated at 80 °C for 45 min, and by 0.297 and 0.839 after being heated at 90 °C for 30 min, for 5% WPI-5% Xyl and 10% WPI-5% Xyl, respectively. The absorbance increase reflected that MRPS was formed in certain extent.

The concentrations for WPI were 5% and 10% (g/mL), respectively; the concentration for Xyl was 5% (g/mL). The mixtures of WPI and Xyl were heated at 80°C and 90°C, respectively, for 0, 15, 30, and 45 min. The development of the Maillard reaction between WPI and Xyl was also monitored by using SDS-PAGE (Fig. 2).

The band at the lowest part of the gel was alpha-lactalbumin, with molecular weight of 14 kDa, and the higher one was beta-lactoglobulin with the molecular weight of 17 kDa [21]. After heating, new bands between 31 kDa-43 kDa and smear bands were observed on the SDS-PAGE, which demonstrated the development of MR. Based on the results of absorbance change and SDS-PAGE, the heating conditions for preparing WPI-Xyl MRPs film were chosen as 80 °C for 45 min or 90 °C for 30 min.

TABLE II. BROWNING DEVELOPMENT OF WPI AND XYL SYSTEM DURING HEATING PROCESS

WPI:Xyl (w:w, %)	Abs (450 nm)				
	0	15	30	45	60 (min)
(80°C)					
5 : 5	0.110±0.001 ^a	0.111±0.005 ^a	0.131±0.010 ^{bc}	0.142±0.001 ^c	0.173±0.001 ^d
10 : 5	0.156±0.004 ^a	0.166±0.003 ^a	0.192±0.005 ^b	0.241±0.003 ^c	0.346±0.012 ^d
(90°C)					
5 : 5	0.105±0.004 ^a	0.142±0.002 ^b	0.244±0.008 ^c	0.402±0.002 ^d	0.663±0.005 ^e
10 : 5	0.156±0.004 ^a	0.247±0.006 ^b	0.528±0.043 ^c	0.995±0.002 ^d	1.671±0.061 ^e

Concentrations for WPI (whey protein isolate) was 5% and 10% (g/mL), respectively, and for Xyl (Xyl) was 5% (g/mL), and the solutions were heated at 80°C or 90°C. Data were presented by mean \pm SD (n=3), and the means in the same row with different letters had significant difference (p<0.05).

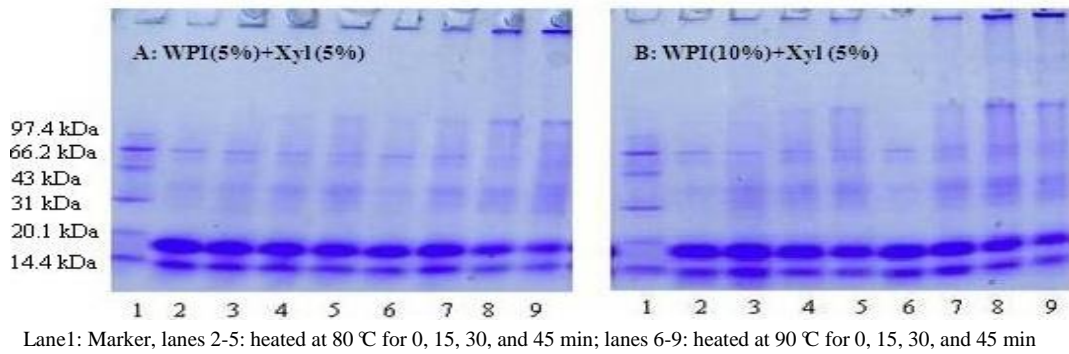


Figure 2. SDS-PAGE of WPI (A: 5%, B: 10%) and Xyl (5%) heated at different temperatures and times

C. Physical and Mechanical Property of WPI-Xyl MRP Film

Increase of Xyl concentration could affect the mechanical property of WPI-Xyl film prepared under both 80°C and 90°C. A decrease of puncture strength from 13.6 N/mm to 6.2 N/mm was observed, while the puncture deformation of the WPI-Xyl MRPs film was not significantly changed. Increase of the Xyl concentration could lower tensile strength of the film, but no significant change was observed for elongation strength (TABLE III).

Xyl was used to form film with chitosan, which consequently led to increase of darkness, decrease of relative amount of free amino groups, and increase of insoluble matter of the chitosan film. Chitosan-Xyl film that contained 10% (g/mL) Xyl showed higher tensile

strength, while further addition of Xyl was related to the decrease of film tensile strength [22].

WVP of WPI-Xyl film was increased as the Xyl concentration in the film was increased (Fig. 3), and the heating temperature did not significantly influence the WVP of films. The results might be related to more –OH when Xyl concentration was high in the system.

Increase of Gly concentration could affect the mechanical property of WPI-Xyl film prepared under both 80°C and 90°C. A decrease of puncture strength from 13.5 N/mm to 8.3 N/mm was observed, while the puncture deformation of the WPI-Xyl MRPs film was not significantly changed. Increase Gly concentration in the films could lower tensile strength of the film, but would not affect elongation strength (TABLE III). The effect of Gly concentration on WPI-Xyl film was similar to its effect on WPI film.

TABLE III. MECHANICAL PROPERTIES OF WPI-XYL MRP FILM PREPARED WITH DIFFERENT XYL CONCENTRATIONS

WPI:Xyl:Gly (w:w:v, %)	PS (N/mm)		PD (mm)		TS (N/mm ²)		E (%)	
	80°C	90°C	80°C	90°C	80°C	90°C	80°C	90°C
Change of Xyl conc.:								
10 : 2.5 : 5	13.6±0.8 ^a	14.3±0.6 ^a	4.44±0.38 ^a	3.06±0.15 ^a	1.73±0.19 ^a	1.49±0.15 ^a	61±8 ^a	65±24 ^a
10 : 5.0 : 5	8.5±1.2 ^b	8.5±0.9 ^b	3.95±0.25 ^a	3.00±0.11 ^a	1.10±0.24 ^{ab}	0.91±0.13 ^{ab}	70±7 ^a	45±10 ^a
10 : 7.5 : 5	6.2±0.3 ^c	5.7±0.1 ^c	4.00±0.12 ^a	2.84±0.27 ^a	0.66±0.02 ^b	0.69±0.09 ^b	64±7 ^a	68±12 ^a
Change of Gly conc.:								
10 : 5 : 4	13.5±0.2 ^a	10.3±0.3 ^a	4.25±0.40 ^a	3.74±0.38 ^a	2.11±0.12 ^a	1.69±0.22 ^a	39±4 ^b	45±17 ^a
10 : 5 : 5	10.9±1.1 ^b	8.3±0.6 ^b	3.84±0.17 ^a	3.54±0.37 ^a	1.36±0.06 ^b	1.14±0.02 ^b	50±4 ^b	44±4 ^a
10 : 5 : 6	8.3±0.2 ^c	6.5±0.5 ^c	4.02±0.34 ^a	3.73±0.33 ^a	1.13±0.07 ^c	0.77±0.11 ^c	45±4 ^b	55±9 ^a

PS: Puncture strength, PD: Puncture deformation, TS: Tensile strength, E: Elongation at break. WPI:Xyl:Gly: the mixtures of WPI, Xyl, and Gly were prepared with concentration(s) of 10% (g/mL) for WPI; 2.5%, 5.0%, and 7.5% (g/mL) for Xyl; 4%, 5%, and 6% (mL/mL) for Gly, respectively. Data were presented by mean ± SD (n=3), and the means in the same column with different letters had significant difference (p<0.05).

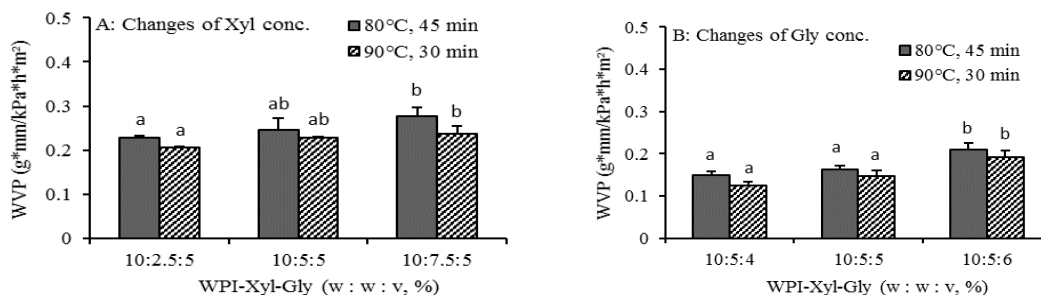


Figure 3. WVP of WPI-Xyl MRPs film prepared with different Xyl and Gly concentrations.

WPI:Xyl:Gly: the mixtures of WPI, Xyl, and Gly were prepared with concentration(s) of 10% (g/mL) for WPI; 2.5%, 5.0%, and 7.5% (g/mL) for Xyl (A); 4%, 5%, and 6% (mL/mL) for Gly (B), respectively. The column values with different letters had significant difference (p<0.05, n=3)

D. Comparisons of WPI and WPI-Xyl MRP Film

Based on previous optimization experiments, the WPI film was prepared at the following condition: 10% (g/mL) WPI, 10% (mL/mL) Gly, and heated at 80 °C for 30 min; the WPI-Xyl MRPs film was prepared at: 10% (g/mL) WPI, 5% (g/mL) Xyl, 5% (mL/mL) Gly, and heated at 80 °C for 45 min or 90 °C for 30 min. Following was comparisons of WPI and WPI-Xyl MRP film.

As shown in TABLE IV, thickness of WPI film was 0.15, while The WPI-Xyl MRPs films were thicker than WPI film. The film absorbance was 0.103 for WPI film and about 0.140 for WPI-Xyl MRPs film. Besides, puncture strength and tensile strength were about 16

N/mm and 1.7 N/mm², respectively, for WPI-Xyl MRPs film, and the value were 1.7 times and 2.4 times of that of WPI film, respectively. The WPI-Xyl film had similar puncture deformation with WPI film, while the elongation percent significantly decreased. The results indicated that WPI-Xyl MRPs film had better mechanical property.

The WVP of WPI film was 0.501 g mm/kPa h m², and the value for WPI-Xyl MRPs film was about 0.380 g mm/kPa h m². The WVP of WPI-Xyl MRPs film was 24% less than WPI film (Fig. 4), and was related to less -OH in MRPs system. MRPs film might be helpful in protecting walnut from rancidity, since their water barrier performance was better.

TABLE IV. MECHANICAL PROPERTIES OF WPI AND WPI-XYL MRP FILMS

WPI:Xyl:Gly (w:w:v, %)	Physical property		Mechanical property			
	Thickness (mm)	Abs (550nm)	PS (N/mm)	PD (mm)	TS (N/mm ²)	E% (%)
(80°C, 30 min) 10 : 0 : 10	0.15±0.01 ^a	0.103±0.005 ^a	9.0±0.3 ^a	3.58±0.15 ^a	0.74±0.05 ^a	81±13 ^a
(80°C, 45 min) 10 : 5 : 5	0.18±0.01 ^b	0.133±0.004 ^b	15.5±0.3 ^b	3.45±0.29 ^a	1.61±0.09 ^b	42±3 ^b
(90°C, 30 min) 10 : 5 : 5	0.18±0.01 ^b	0.156±0.007 ^c	16.1±1.1 ^b	3.47±0.28 ^a	1.73±0.07 ^b	41±2 ^b

PS, puncture strength; PD: puncture deformation TS: tensile strength; E: elongation at break. WPI:Xyl:Gly: the mixtures of WPI, Xyl, and Gly were prepared at the ratios of 1:0:1 and 1:0.5:0.5, and the concentrations of 10% (g/mL) WPI, 0 or 5% (g/mL) Xyl, and 10% or 5% (mL/mL) Gly, respectively. Data were presented by mean ±SD (n=3), and the means in the same column with different letters had significant difference (p<0.05).

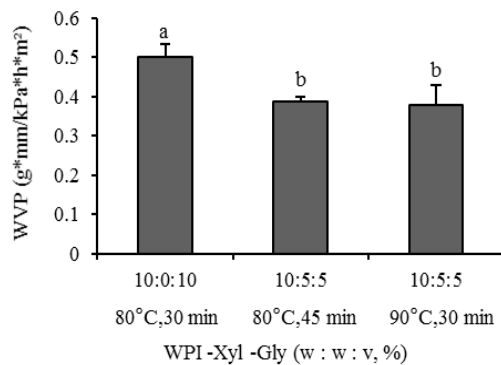


Figure 4. WVP of WPI and WPI-Xyl MRP films

WPI:Xyl:Gly: the mixtures of WPI Xyl, and Gly were prepared at the ratio of 1:0:1, 1:0.5:0.5, and their concentrations were 10% (g/mL) for WPI, 0, 5% (g/mL) for Xyl, and 10%, 5% (mL/mL) for Gly, respectively. The column values with different letters had significant difference (p<0.05, n=3).

E. Analysis of Walnut Rancidity

The acid value of walnut during the storage was presented in Fig. 5. Acid value of all walnut stored at 50 °C, RH 80% began to increase significantly after 20 days compared with those stored at room temperature (28 °C), and showed faster increase than that of WPI-Xyl MRPs coated walnut.

Vegetable lipids that contain unsaturated fatty acids tend to decompose in contact with the atmosphere or lipases and release their fatty acid constituents. The free fatty acids (FFA) are extremely susceptible to oxidation.

The process induces the unpleasant smell and taste. Therefore, the acid value that reflects the content of FFA is frequently determined as quality indicators [23]. Similar acid value of walnut with WPI coatings and without coatings indicated that WPI coatings did not effectively alleviate the degree of rancidity. Ref. [8] observed the ineffectiveness of WPI film on the oxidative rancidity of walnut during the storage at 37°C for 40 days. They claimed that the shrinkage of the coating on the walnut surface was likely a reason. MRPs coating could help to delay the rancidity of walnut, as was shown by lower acid value than uncoated group. There was less Gly in MRPs film, which led to less serious shrinkage of walnut and lower water permeability of film. It helped to improve the performance of films and help to delay the rancidity of walnut. Besides, the participation of Xyl could also contribute to the effect of MRPs coating.

The TBARS values of walnut oil were determined and presented by absorbance at 520 nm. As shown in Fig. 6, the TBARS value was about 0.15 at the beginning. The values of walnut without coating or with WPI coating were 0.19 and 0.18, were significantly higher than those with MRPs coating, whose TBARS values was about 0.16 after the storage.

The TBARS value is a useful indicator for screening the lipid peroxidation products, especially malondialdehyde (MDA) [24]. Coatings made from WPI-Xyl MRPs were more helpful than from WPI in

delaying the increase of lipid peroxidation in walnut and protecting walnut from rancidity.

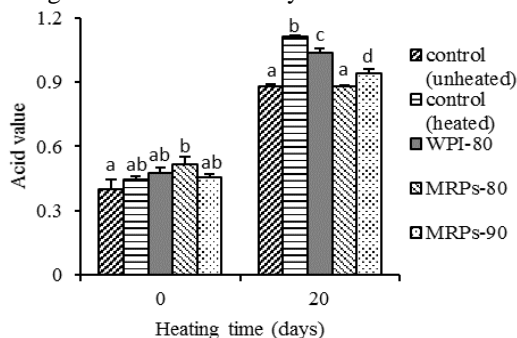


Figure 5 Acid value of walnut coated with WPI-Xyl MRPs

“Control (unheated)”: untreated walnut and stored at room condition (28°C); “Control (heated)”: untreated walnut and stored at 50°C, RH 80%; “WPI-80”: WPI coated walnut and stored at 50°C, RH 80%; The film solution contained 10% (g/mL) WPI and 10% (mL/mL) Gly, and was heated at 80°C for 30 min; “MRPs -80” and “MRPs-90”: MRPs coated walnut and stored at 50°C, RH 80%; The film solution contained 10% (g/mL) WPI, 5% (g/mL) Xyl, 5% (mL/mL) Gly, and was heated at 80°C for 45 min or 90°C for 30 min; data at the same block with different letters had significant difference ($p < 0.05$, $n=3$).

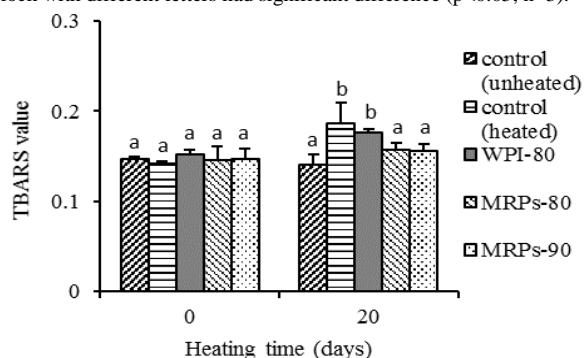


Figure 6. TBARS values of walnut coated with WPI-Xyl MRPs

“Control (unheated)”: untreated walnut and stored at room condition (28°C); “Control (heated)”: untreated walnut and stored at 50°C, RH 80%; “WPI-80 coated”: WPI coated walnut and stored at 50°C, RH 80%; The film solution contained 10% (g/mL) WPI and 10% (mL/mL) Gly, and was heated at 80°C for 30 min; “MRPs -80 coated” and “MRPs-90 coated”: MRPs coated walnut and stored at 50°C, RH 80%; The film solution contained 10% (g/mL) WPI, 5% (g/mL) Xyl, 5% (mL/mL) Gly, and was heated at 80°C for 45 min or 90°C for 30 min; data with the different letters had significant difference ($p < 0.05$).

IV. CONCLUSION

WPI film and WPI-Xyl MRPs film with good physical and mechanical properties could be prepared under optimum conditions of 10% (g/mL) WPI, 10% (mL/mL) Gly, heated at 80 °C for 30 min; and 10% (g/mL) WPI, 5% (mL/mL) Gly, 5% (g/mL) Xyl, heated at 80 °C for 45 min or 90 °C for 30 min, respectively. Penetration strength and elongation strength were higher, while water vapor permeability was lower for WPI-Xyl MRPs film than for WPI film.

WPI film coatings could help to protect walnut from rancidity, while the WPI-Xyl MRPs film coatings performed effectively on delaying the rancidity of walnut, as evidenced by decreased acid value and TBARS values. The walnut coating with WPI-Xyl MRPs film, prepared

from WPI MR products, demonstrated good protective effect against walnut peroxidation. MR could be used as an effective method to improve film property and coating effect of WPI.

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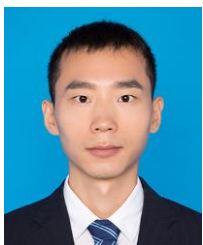
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published papers co-authored with his name: “Characteristic fluorescence of bovine serum albumin and different sugar Maillard reaction systems” (Beijing, China, *Journal of Food Safety and Quality*, 2015); “Glycation of bovine serum albumin with monosaccharides inhibits heat-induced protein aggregation” (RSC Advances, 2016). His research has been focused on protein-saccharide Maillard reaction, and protein-polyphenol interaction and nanoparticle.



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