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Assessment of the physical, mechanical, and moisture-retention properties of pullulan-based ternary co-blended films

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ABSTRACT

Multi-component substances made through direct blending or blending with co-drying can form films on the surfaces of intermediate moisture foods (IMFs), which help retain moisture and protect food texture and flavor. An IMF film system based on pullulan, with glycerol serving as the plasticizer, was studied using alginate and four different types of polysaccharides (propyleneglycol alginate, pectin, carrageenan, and aloe polysaccharide) as the blend-modified substances. The physical, mechanical, color, transparency, and moisture-retention properties of the co-blended films with the polysaccharides were assessed. A new formula was established for the average moisture retention property, water barrier, tensile strength, elongation at break, and oxygen barrier property of the ternary co-blended films using the Design Expert software. The new model established for moisture content measurement used an indirect method of film formation on food surfaces by humectants, which should expedite model validation and allow a better comprehension of moisture transfer through edible films.

1. Introduction

As an intermediate moisture food (IMF), the moisture of vacuum-freeze-dried fiddleheads is between 10% and 15%, and the water activity is between 0.5 and 0.6. Moreover, vacuum freeze-drying is one of the oldest methods of preserving fiddleheads (Karel & Heidelbaugh, 1973; Miranda, Berna, Bon, & Mulet, 2011; Pavey & Schack, 1969). The main components of vacuum-freeze-dried fiddleheads include protein, carbohydrates, lipids, and other humectants (DeLong et al., 2013). Compared with traditional dried or high-moisture fiddleheads, intermediate moisture vacuum-freeze-dried fiddleheads have many advantages. The first benefit is a lower energy consumption during processing and transportation compared with dried, refrigerated, frozen, and canned foods. Vacuum freeze-drying also lessens the destruction of the fiddleheads during processing compared with comparable stronger methods, such as dehydration and heat treatment. The low water activity (0.5–0.6) of vacuum-freeze-dried fiddleheads will control microorganisms and better maintain their preservation at room temperature (shelf life: 1/2 year to 1 year); Finally, fiddleheads prepared in this manner are mild taste and easy to eat because they are directly edible without heating or rehydrating (Brimelow, 1985). However, vacuum-freeze-dried fiddleheads still present several problems, such as being affected by dry or wet conditions, structural destruction, and loss of flavor, all of which significantly influence the nutritional value and the consumer acceptance (Hierro, de la Hoz, & Ordonez, 2004; Rao, Rocca-Smith, & Labuza, 2012).

The public perception of the potential toxicological properties of food additives has also limited the widespread development of vacuum-freeze-dried fiddleheads for human consumption. Recent applications have focused on developing vacuum-freeze-dried fiddleheads for use in space programs and the military. The addition of humectants to fresh vegetables or fruits can reduce the water activity into ranges that allow their storage without refrigeration (Carrasco & Cisneros-Zevallos, 2002; Gliemmo, Campos, & Gerschenson, 2004; Rojas & Gerschenson, 2001). The addition of humectants and plasticizers is the basic method for controlling the moisture and structure of vacuum-freeze-dried fiddleheads. The common humectants used are sugars (such as glucose, fructose, fructose syrup, glucose syrup, and polysaccharides), polyhydroxy compounds (such as sorbitol and glycerol), and salts (Gliemmo et al., 2004; Rojas & Gerschenson, 2001; Sritongtae, Mahawanich, & Duangmal, 2011).

The study of polysaccharides as humectants started years ago. Ternary blends of polysaccharide solutions prepared through the co-drying method can have synergistic effects, that endow the composite with superior functional features, including increasing

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the viscosity of a single polysaccharide solution, improving the gelation function, and increasing the barrier and mechanical performance of the film formed (Ohashi et al., 1991; Salvador, Sanz, & Fiszman, 2001). Over the years, the study of blending polysaccharides has mainly focused on the simple physical mixing and synergy of two polysaccharides. In addition, few studies have focused on the characteristics of polysaccharide solutions formed by a ternary system (Damasio, Fiszman, Costell, & Durán, 1990; Salvador et al., 2001). Generally speaking, the electrostatic interactions between the different large molecules in polysaccharides are the reason for which blended systems perform better. Ternary polysaccharides produced through the co-drying method can be used as food humectants in IMF, as they improve the preservation and manufacturing properties, by increasing the water barrier, mechanical strength, and appearance acceptance. The traditional testing of the characteristics of humectants mainly focused on the moisture variation under specific conditions, thus establishing the relationships between the humectant effectiveness and time (Hou et al., 2012; Yang et al., 2010).

This article assesses the humectant effectiveness of the ternary blending of polysaccharides by co-drying on vacuum freeze-dried fiddleheads. Furthermore, this article studies the film characteristics of polysaccharides on the surface of the fiddleheads. These results were used to establish a correlation model between the film characteristics of water barrier, oxygen barrier, mechanical strength and moisture effectiveness. This measurement will be used to fully evaluate the humectant characteristics of ternary blended polysaccharides, including their water retention, anti-moisture absorption, mechanical property, and improvements in their appearance acceptance.

2. Materials and methods

2.1. Materials

Following emergence and before 10 cm of growth, the fiddleheads of *Matteuccia struthiopteris* were harvested from Changbai Mountain in northeast China during 2012. The crosiers were either refrigerated or maintained on ice until they were cleaned and then stored at 4 °C. All of the tissue samples were freeze-dried and stored at 4 °C until further analysis.

The pullulan (PUL) and aloe polysaccharide (ALO) were obtained from Jiangnan University (Wuxi, China). The reagents used for film preparation and testing (glycerol, alginate (ALG), propyleneglycol alginate (PGA), pectin (PEC), carrageenan (CAR), magnesium nitrate, sodium hydroxide, and anhydrous calcium chloride) were purchased from Fisher Scientific Ltd. (Shanghai, China).

2.2. Co-dried PUL/ALG and PUL/ALG/polysaccharides blended preparation

Solutions of 50.00 g/L PUL and 50.00 g/L ALG were prepared in distilled water at 60 °C, and 0.05 (g/g PUL) polysaccharides (PGA, PEC, CAR, and ALO) were then added to the solutions. The concentration of the solution was adjusted to achieve a viscosity of less than 500 cps, and the pH was adjusted to 7.0 using 2 M NaOH. The spray drying was performed using a BUCHI B-290 Mini Spray Dryer with an atomizer speed of 15,000 rpm, an inlet temperature of 200 °C, and an outlet temperature of 95 °C. The resultant dried powder (PUL/ALG/PGA-CO, PUL/ALG/PEC-CO, PUL/ALG/CAR-CO, and PUL/ALG/ALO-CO) was the neutral ternary polysaccharide co-dried powder.

The pH was adjusted to 7.0 with a 50.00 g/L PUL solution and a 50.00 g/L ALG solution, and the blend-modified powder was adjusted to obtain a binary neutral co-dried powder (PUL/ALG-CO).

2.3. PUL/ALG film and PUL/ALG/polysaccharides film preparation

The different powders obtained (PUL/ALG, PUL/ALG-CO, and PUL/ALG/polysaccharides, PUL/ALG/polysaccharides-CO) were slowly added into stirring deionized water. The stirring was maintained for 2 h to uniformly disperse the powders into the aqueous solution to obtain a stable solution of 50.00 g/L. The solution, the pH of which was adjusted to 7.0 with 2 M NaOH, was prepared and heated in a 250-mL Erlenmeyer flask for 30 min in a water bath at 80 °C. The emulsions were then cooled in an ice bath. Glycerol (0.30 g/g PUL) was added in the amount required to achieve the desired final film composition, and the film-forming solutions were de-gassed under vacuum of 0.09 MPa for 10 min using a vacuum pump (SHZ-D(III), Yuhua Instruments Co. Ltd., Gongyi, China).

The films were prepared by placing the specific amount of the degassed film-forming solution or emulsion that would provide 3.00 g of total solids on a smooth high-density polyethylene (HDPE) casting plate resting on a leveled granite surface, because it is not hygroscopic and has good resistance in WVP. Additionally, HDPE has good chemical stability, which means that it is insoluble in any organic solvent, and has good resistance to corrosion by acids, bases, and all types of salts (Araujo, Mano, Teixeira, Spinace, & De Paoli, 2010). The films were dried for approximately 20 h in the oven at 53% relative humidity (RH) and 25 °C. All of the dried films could be peeled intact from the casting surface. The films used for water barrier property assessment and mechanical testing were conditioned at 53 ± 1% RH and 25 ± 1 °C by placing them in a desiccator containing a saturated solution of $Mg(NO_3)_2{\cdot}6(H_2O)$ for at least 72 h. The compositions of the different polysaccharide films, including the PUL/ALG, PUL/ALG/PGA, PUL/ALG/PEC, PUL/ALG/CAR, and PUL/ALG/ALO films prepared from direct blended polysaccharide powder and the PUL/ALG-CO, PUL/ALG/PGA-CO, PUL/ALG/PEC-CO, PUL/ALG/CAR-CO, and PUL/ALG/ALO-CO films prepared from the neutral co-dried polysaccharide powders, are shown in Table 1.

2.4. Film thickness measurement

The film thickness was determined using a digital micrometer (High-Accuracy Digimatic Digital Micrometer, USA). The film strips were placed between the jaws of the micrometer, and the gap was reduced until the friction was minimal. The mean thickness (m) of the films was measured from the average of numerical values at 10 locations.

2.5. Water vapor permeability (WVP) measurement

The water vapor transmission rate (WVTR) of the film specimens were measured according to a modified ASTM E96 method (ASTM, 2002; Gennadios, McHugh, & Weller, 1994; Ou, Wang, Tang, Huang, & Jackson, 2005). Glass cups with a diameter of 3.00 cm and depth of 4.00 cm were used for this measurement. To maintain 0% RH in the cup headspace, 3.00 g of dried $CaCl_2$ was added to the cup, and the film was then sealed over the rim of the cup by applying molten paraffin. The cups were placed in hermetically sealed jars maintained at 20 °C and 100% RH. The RH was maintained by placing 1000 mL of water in the bottom of the jar. The cups were weighed every 12 h for a period of one week. The amount of water that permeated the films was determined from the weight gain of the cups. The WVTR and WVP were calculated using the following equations:

$$\mathrm{WVTR} = \Delta w/\Delta t \times A, \quad \mathrm{WVP} = \mathrm{WVTR} \times L/\Delta p$$

where WVTR is expressed in g/h m^2, $\Delta w/\Delta t$ is the rate of water gain in g/h, A is the exposed area of the film in m^2, L is the mean

Table 1
The WVP, TS, *E*%, OP, and moisture retention ability of the blend-modified polysaccharide edible films.[a]

Blending method	Blend-modified polysaccharide edible films	WVP (g mm/m^2 h kPa) (X1)	TS (MPa) (X2)	*E*% (X3)	OP (cm^3 mm/m^2 day kPa) (X4)	Average moisture retention value (RH = 30%) (Y1)	Average moisture retention value (RH = 80%) (Y2)
Direct addition	PUL/ALG (50.00 g/L)	1.69 ± 0.08AB	69.34 ± 4.45C	5.71 ± 0.16FG	326.58 ± 12.07D	10.84 ± 2.09E	16.39 ± 1.40C
	PUL/ALG/PGA (50.00 g/L)	1.61 ± 0.03A	91.47 ± 8.12A	7.89 ± 0.33D	311.38 ± 8.82D	10.93 ± 2.04D	16.33 ± 1.36C
	PUL/ALG/PEC (50.00 g/L)	1.80 ± 0.03B	71.32 ± 7.22C	7.66 ± 0.29D	324.72 ± 6.16D	10.77 ± 2.11F	16.46 ± 1.41D
	PUL/ALG/CAR (50.00 g/L)	1.93 ± 0.07C	70.11 ± 6.01C	6.19 ± 0.18E	357.65 ± 12.96C	10.63 ± 2.13G	16.51 ± 1.43D
	PUL/ALG/ALO (50.00 g/L)	2.78 ± 0.09D	60.04 ± 8.74C	2.11 ± 0.12I	498.07 ± 9.50A	9.33 ± 2.45H	17.82 ± 2.10E
	PUL/ALG-CO (50.00 g/L)	1.04 ± 0.03E	91.20 ± 6.24B	5.96 ± 0.21EF	289.64 ± 7.76E	11.05 ± 1.97C	16.18 ± 1.28B
	PUL/ALG/PGA-CO (50.00 g/L)	0.51 ± 0.05G	103.68 ± 3.63A	46.35 ± 1.12A	197.65 ± 11.82G	12.18 ± 1.39A	15.29 ± 0.63A
Co-dried addition	PUL/ALG/PEC-CO (50.00 g/L)	1.11 ± 0.04EF	95.86 ± 5.55A	13.68 ± 0.23B	216.40 ± 5.58G	11.62 ± 1.63B	15.42 ± 0.69A
	PUL/ALG/CAR-CO (50.00 g/L)	1.20 ± 0.08F	97.17 ± 6.08A	12.37 ± 0.12C	239.76 ± 7.35F	11.56 ± 1.69B	16.12 ± 1.23B
	PUL/ALG/ALO-CO (50.00 g/L)	1.92 ± 0.03C	68.12 ± 4.63C	4.16 ± 0.06H	401.13 ± 29.24B	9.50 ± 2.28H	17.75 ± 2.22E

[a] The values are the means ± the standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

thickness of the film specimens in m, and Δp is the difference between the partial water vapor pressure on the two sides of the film specimens in Pa. The water vapor pressure on the high-stream side of the film was 2.34 kPa (i.e., saturated water vapor pressure at 20 °C), whereas that of the low-stream side was assumed to be zero.

2.6. Measurement of mechanical properties

Before testing their mechanical properties, the film samples were equilibrated for one week at 25 °C and 53% RH. Two mechanical parameters of the films, namely the tensile strength (TS) and the elongation at break (*E*%), were determined using a texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK). The film samples were cut into 10-mm-wide and 800-mm-long strips using a sharp razor blade. Ten samples of each film type were tested. The tensile properties of the films were measured according to the ASTM standard method D882-02 (de Carvalho & Grosso, 2004). The TS was calculated based on the original cross-sectional area of the test specimen using the equation TS = *F*/*A*, where TS is the tensile strength (MPa), *F* is the force (N) at maximum load, and *A* is the initial cross-sectional area (m^2) of the film specimen. The *E*% value was calculated by dividing the extension-at-break of the specimen by the initial gauge length and multiplying by 100.

2.7. Oxygen barrier property (OP) measurement

A 60-mL jar was filled with 30.0 mL of fresh soybean oil, covered with different films, sealed using a bungee cord, and stored at a controlled temperature (60 °C) for 10 days. The peroxide value of the soybean oil samples was determined through sodium thiosulfate titration (Tang, Jiang, Wen, & Yang, 2005). All of the tests were conducted in triplicate.

2.8. Measurement of moisture retention ability

The moisture retention properties were measured in this study using multiple variable differentials. First, 100.00 g of uniformly mixed vacuum-freeze-dried fiddleheads were weighed and equilibrated for 48 h at 22 ± 1 °C and (60 ± 2)% RH. This sample was then separated into two groups (50.00 g in each group). One group was prepared through a uniform spraying of blend-modified polysaccharide and glycerin at the proportion of 5.00 g/100 g of vacuum-freeze-dried fiddleheads. The other group was prepared by spraying the equivalent amount of distilled water as the reference. The two groups were equilibrated for 48 h at 22 ± 1 °C and (60 ± 2)% RH (Sun et al., 2006; Yang et al., 2010). For the measurement of the changes in the aqueous ratio of the vacuum freeze-dried fiddleheads during the moisture removal and absorption processes, the two groups were equilibrated in a chamber with a constant temperature (22 ± 1 °C) and humidity (RH of (A ± 2)%) for 48 h. Four groups of samples were prepared in 70-mm weighing bottles with approximately 6.00 g in each group. The aqueous ratios of the samples from two groups were tested using the oven method (deviation ≤ 0.1%), and the average of the tested values was defined as the original aqueous ratio of the sample. The other two groups were maintained in a sulfuric acid desiccator with a relative humidity of 30 ± 2% to observe the moisture removal process and (80 ± 2)% RH to observe the moisture absorption process. The sulfuric acid desiccators were maintained in a chamber with constant temperature (22 ± 1 °C) and humidity to study the changes in the aqueous ratios during moisture removal and the absorption process of vacuum-freeze-dried fiddleheads. Equilibration of the vacuum-freeze-dried fiddleheads was generally achieved after 48 h in the different environments with different relative humidities. Therefore, the observations

associated with the aqueous ratio changes during moisture removal and the absorption process were performed over a period of 5 days. The samples were weighed and the time was recorded every 4 h during the first three days (no less than three times per day). During the last two days, the samples were weighed once per day. At each time point, the aqueous ratio difference (compared with the original aqueous ratio) and the weight difference were recorded.

Using the data from the two groups of samples, a curve of aqueous ratio over time was plotted. Utilizing the slope of the water content curve at the early stage and its comparison to the equilibrium aqueous ratio at the later stage, the moisture retention property of vacuum-freeze-dried fiddleheads with the addition of humectants (and the appropriate reference) was compared and evaluated (Sun et al., 2006).

2.9. Color and film transparency measurement

The color of the film was determined using an UltraScan Pro1166 (Hunterlab, USA) and expressed as L^*, a^*, and b^*. The film transparency was determined according to the method developed by Ubonrat Siripatrawan using a UV spectrophotometer (UV-2802H, Unico, Shanghai) and calculated according to the following equation (Siripatrawan & Harte, 2010):

$$T = \log T_{600}/\text{thickness}$$

where T_{600} is the fractional transmittance at 600 nm and thickness is the film thickness (mm). According to this equation, high values of T indicate low transparency and a higher degree of opacity. All of the tests were conducted in triplicate.

2.10. Experimental design and statistical analysis

Three independent property measurements were conducted at different times to obtain three experimental replications ($n = 3$) for all PUL-based films. The data were analyzed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The comparisons between the PUL-based films were made through an analysis of variance (ANOVA) with post hoc comparisons of the mean values using Duncan's multiple-range test. The differences between the mean values were considered significant at $p < 0.05$. The effects of the four independent variables, specifically the WVP, TS, *E*%, and OP measurements, on the moisture retention ability of the blend-modified polysaccharide edible films were studied using a four-factor CCD (Central Composite Design).

The 10 blend-modified polysaccharide samples were established based on the CCD with four independent variables. Multiple regression analysis was applied to predict the linear, quadratic, and interaction terms of the independent variables in the RSM (Response Surface Method). A regression analysis was performed to estimate the response function as a polynomial model: $Y = b_0 + \sum b_i X_i$, where Y is the response calculated by the model, b_0 is a constant, and b_i is the linear coefficient. The data were modeled through multiple regression analysis based on a stepwise analysis. Only those variables that were found to be significant at the $p < 0.05$ level were selected for the model construction. The significant terms in the model for each response were found through an analysis of variance (ANOVA). The acceptability of the model was analyzed, accounting for the lack of fit, adequate precision, and pure error. The experimental data were compared with the fitted values predicted by the models to verify the suitability of the regression models. Each experiment was repeated in triplicate, and the mean values, including the pooled standard error of the mean (SEM), were then determined.

3. Results and discussion

3.1. Properties of co-blended polysaccharide films

Due to the beneficial compatibility with the polysaccharide added, a good mixed gel system was formed, and polysaccharide films with satisfactory smoothness and transparency were obtained. The quantity of dry matter in the film liquid per unit of flat area was maintained constant throughout the experiment to keep the film thickness constant (0.10 ± 0.01 mm), which ensured minimal influence of the film thickness when measuring the film properties (Gennadios et al., 1994).

The co-blended polysaccharide solution determines the co-blended film properties to some extent. The measurement results for the properties of the binary and ternary co-blended systems, including WVP, TS, *E*%, and OP, after the addition of the polysaccharides are shown in Table 1. From these results, a better performance, based on improved water barrier properties, mechanical properties, and OP, was found with the PUL/ALG-CO binary co-blended film after blend-modification compared with the PUL/ALG film obtained through direct addition.

The WVP of the ternary co-blended film formed by the direct addition of PEC, CAR, and ALO was higher than that of the PUL/ALG film, whereas the direct addition of PGA increased the water barrier property. The TS and *E*% of the ternary co-blended film with the direct addition of ALO were lower than those of the PUL/ALG film, whereas the TS and *E*% of the ternary co-blended film with the addition of PGA, PEC, and CAR were higher than those of the binary films. Contrary to the negative influence of CAR and ALO on the OP, the OP of a ternary co-blended film with the direct addition of PGA and PEC would be better than that of a binary co-blended film. As shown in Table 1, the additions of PGA, PEC, and CAR by co-drying significantly improved the water barrier property, which is the same effect as those of the TS, *E*%, and OP of the ternary co-blended film with the addition of ALO. Among all of the samples, the WVP of the ternary co-blended PUL/ALG/PGA-CO film obtained with the co-drying modification was decreased by 69.8% compared with that of the PUL/ALG film. Generally speaking, the compounds formed by different polysaccharides exhibited stronger functional properties, such as the gel property and the aggregative property. Three independent gel systems were formed because the interactions of the different components of different complex types of polysaccharides were reduced due to the electronic incompatibility of polysaccharides (Zaleska, Ring, & Tomasik, 2000). However, the electronic compatibility of the polysaccharides and their interactions increased after modification through co-drying, but the detailed mechanisms underlying the formation of these co-dried films are not completely understood (Hosseini, Rezaei, Zandi, & Ghavi, 2013). Moreover, the surface activity, functional property, and water barrier property of the co-dried powder were significantly improved by this method. Different polysaccharides in ternary co-blended films have different effects on the water barrier properties of the films produced by the direct blending and co-drying modifications due to the different interactions between different polysaccharides. The PGA and PUL/ALG films formed a stronger gel network structure due to their hydrogen bonding and electrostatic interactions, which also resulted in the best water barrier property. In contrast, PEC and PUL/ALG formed a weaker hydrogen bonding network, which resulted in a comparatively poorer water barrier property. CAR has different gel forming stages than the other polysaccharides, resulting in poor compatibility and a comparatively higher WVP. ALO only formed weak, non-covalent bonds with PUL/ALG, resulting in the highest WVP and the worst water barrier property (Villagomez-Zavala et al., 2008).

The addition of PGA, PEC, and CAR significantly improved the film plasticity and mechanical property. The TS of the ternary films

obtained through the addition of polysaccharides by co-drying increased 13.3%, 34.4%, 38.6%, and 13.46%, respectively, compared with those of the films obtained by direct addition. Alternatively, the *E*% of these ternary films with the addition of polysaccharides by co-drying increased 487.5%, 78.6%, 99.8%, and 97.2%, respectively, compared with those of the films obtained through direct addition.

As shown in Table 1, with the exception of ALO, the co-blending modification of PGA, PEC, and CAR significantly improved the OP of the ternary films formed. The oxygen permeability ratio of the ternary co-blended films decreased 39.5%, 33.7%, and 26.6%, respectively, compared with that of the PUL/ALG film. The oxygen permeability of the ternary blend-modified PUL/ALG/PGA-CO, PUL/ALG/PEC-CO, PUL/ALG/CAR-CO, and PUL/ALG/ALO-CO films decreased 36.5%, 33.4%, 33.0%, and 19.5%, respectively, compared with these films made through direct blending.

It can be concluded that the ternary co-drying of the modified polysaccharide films, especially the function of drying-modified PGA on the co-blending property of polysaccharides, can increase the application value of co-blended films to some extent.

3.2. Dehydration and hygroscopicity

Methods with several differentials were utilized to study the moisture removal process of 12 groups of vacuum-freeze-dried fiddlehead samples that were sprayed with 10 different types of blend-modified polysaccharides, glycerin, and a reference compound in a $(22 \pm 1)^{\circ}$C environment with a RH of (30 ± 2)% and then subjected to a moisture absorption process at $(22 \pm 1)^{\circ}$C with a RH of (80 ± 2)%. The aqueous ratios were also measured simultaneously. Curves of the aqueous ratio of the vacuum-freeze-dried fiddleheads as a function of the measuring time were then prepared and are shown in Fig. 1a and b.

At 30% RH, the water content (%) of all vacuum-freeze-dried fiddlehead samples coated with the blend-modified polysaccharides decreased gradually over a period of 120 h, which is quite similar to the behavior obtained with glycerol and the reference. The sequence of the aqueous ratios of the vacuum-freeze-dried fiddleheads (within 120 h) was as follows: PUL/ALG/PGA-CO > PUL/ALG/PEC-CO > PUL/ALG/CAR-CO > PUL/ALG-CO > PUL/ALG/PGA > PUL/ALG > PUL/ALG/PEC > PUL/ALG/CAR > glycerin > PUL/ALG/ALO-CO > PUL/ALG/ALO > reference. The analysis of variance was performed to determine the factors that influence the aqueous ratio of vacuum-freeze-dried fiddleheads, and the results are shown in Table 1. This analysis indicated that the addition time, the type of co-blended polysaccharide added, and its interactions had a significantly different influence on the aqueous rate of the vacuum-freeze-dried fiddleheads. Multiple comparisons regarding the influence of different humectants were performed, and results are shown in Fig. 1a and b. This figure indicates that the aqueous ratios of the vacuum-freeze-dried fiddleheads after the humectant treatment, including PUL/ALG/PGA-CO, PUL/ALG/PEC-CO, and PUL/ALG/CAR-CO, were significantly higher than those of the vacuum-freeze-dried fiddleheads in the other nine treatment groups (Fig. 1a). The co-dried blended ternary polysaccharides are expected to have proportionally more polar residues, which imparts the ability to form hydrogen bonds with water and increase the moisture absorption and retention properties (Gbogouri, Linder, Fanni, & Parmentier, 2004). Therefore, the ternary co-blended polysaccharides, particularly PUL/ALG/PGA-CO, showed good moisture absorption and retention properties. Furthermore, in 30% RH, the best humectant moisturizing property was found with 0.5% PUL/ALG/PGA-CO, followed by 0.5% PUL/ALG/PEC-CO and 0.5% PUL/ALG/CAR-CO. Generally speaking, the three humectants mentioned are suitable for the application of this technique in an environment with lower humidity. A later study will focus on the correlation of the comprehensive performance and moisture retention properties of the films to investigate the feasibility of a new method for moisture retention assessment.

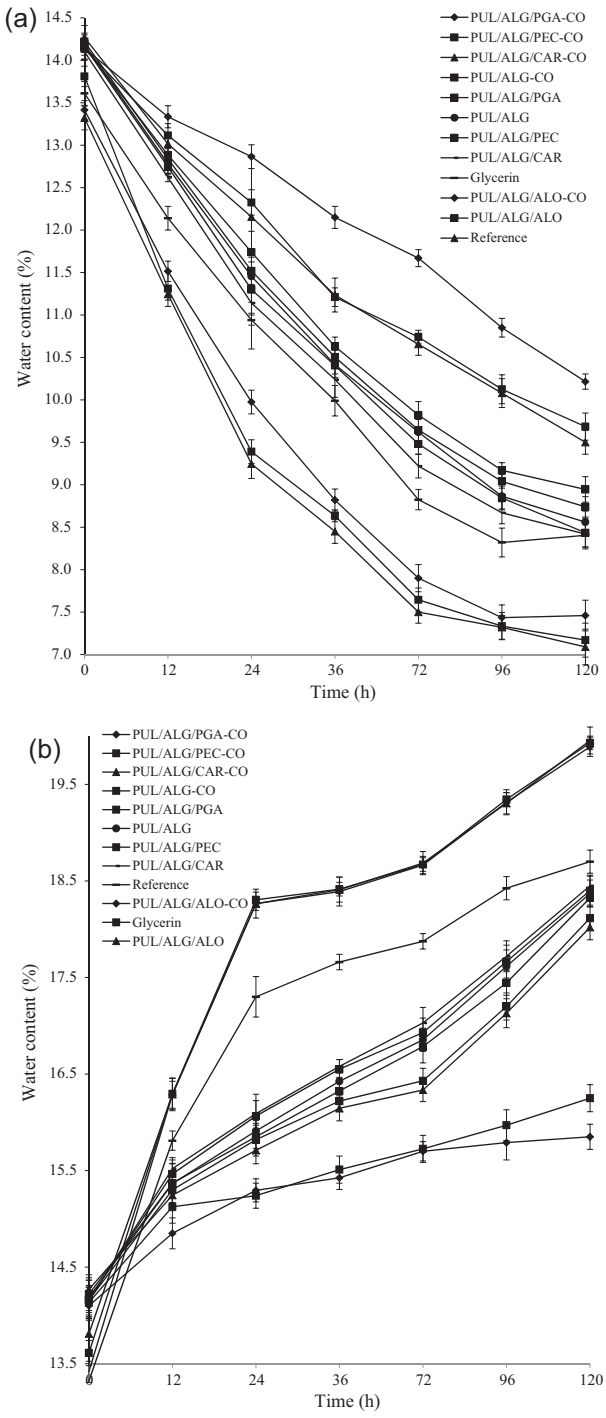

Fig. 1. (a) Dehydration of the blend-modified polysaccharides, glycerin, and the reference. (b) Hygroscopicity of the blend-modified polysaccharides, glycerin, and the reference.

For the typical behavior of water vapor-sensitive hydrophilic polysaccharides, the selection of humectants in an environment with high humidity was stricter, there, the focus should be placed on the products' moisture-resistant properties (Ryu, Kim, Park, Lee, & Lee, 2007; Silhacek & Murphy, 2008). At 80% RH, the weight of the residual moisture in the vacuum-freeze-dried fiddleheads increased during

Table 2
The regression coefficients, R^2, adjusted R^2, and lack of fit for the final reduced models.

Regression coefficient	Average moisture retention values (RH = 30%) (*Y*1)	Average moisture retention values (RH = 80%) (*Y*2)
b_0	13.236	13.361
b_1	0.466	−0.449
b_2	0.003	0.003
b_3	0.012	−0.009
b_4	−0.011	0.011
R^2	0.9530	0.9108
Adjusted R^2	0.9154	0.8395
Lack of fit (*F*-value)	25.3486	12.7691
Lack of fit (*p*-value)	0.0016	0.0078

b_i (i = 1, 2, 3, or 4), the estimated regression coefficient for the main linear effects. 1, WVP (g mm/m^2 h·kPa); 2, TS (MPa); 3, *E*%; 4, OP (cm^3 mm/m^2 day kPa).

the 120 h of the study. The sequence of moisture-retention ability was as follows: PUL/ALG/ALO > glycerin > PUL/ALG/ALO-CO > reference > PUL/ALG/CAR > PUL/ALG/PEC > PUL/ALG > PUL/ALG/PGA > PUL/ALG-CO > PUL/ALG/CAR-CO > PUL/ALG/PEC-CO > PUL/ALG/PGA-CO. The aqueous ratios of vacuum-freeze-dried fiddleheads treated with PUL/ALG/ALO, glycerin, and PUL/ALG/ALO-CO were higher than that of the control sample treated with distilled water, indicating that these three humectants exhibit poor moisture barrier properties. The water-resistance properties of the vacuum-freeze-dried fiddleheads following the addition of PUL/ALG/PGA-CO and PUL/ALG/PEC-CO were significantly higher than those of the other samples in the high-humidity environment (Fig. 1b), which coincides with their amphiphilic structure (Pennick, Chavan, Summers, & Rawlings, 2012). Therefore, under high-humidity conditions, PUL/ALG/PGA-CO and PUL/ALG/PEC-CO are the preferred humectants due to their moisture barrier properties.

3.3. Correlation between WVP, TS, E% and OP, and moisture retention ability

The traditional method for measuring the moisture retention property is too complicated and does not provide exhaustive and accurate results. Therefore, this study was designed to assess the moisture-retention property based on the film properties (water barrier, moisture barrier, and mechanical properties with particular focus on the penetration of aromatic components). Thus, this assessment was more comprehensive. To study the accuracy of this assessment method, a correlative analysis was performed on the properties of ternary co-blended films and their moisture-retention properties as measured by a differential method (Hou et al., 2012; Sun et al., 2006).

The values of water content for each of the blend-modified polysaccharide edible films are shown in Fig. 1a and b. The linear coefficient of WVP (*X*1), TS (*X*2), *E*% (*X*3), and OP (*X*4) for each of the response variables is given in Table 2. The R^2 and adjusted R^2 values for the significant ($p < 0.05$) response surface models varied from 0.9108 to 0.9530 and from 0.8395 to 0.9154, respectively. These values were obtained for the moisture-retention ability at a storage temperature of 22 °C with a RH of 30% or 80% for 120 h. The selected variables yielded the highest values of R^2 and adjusted R^2 for each response variable studied. All of the models were significant for all of the variables (RH = 30% ($p = 0.0016$) and RH = 80% ($p = 0.0078$); (Table 2)). Therefore, the RSM was able to predict most of the variations in the moisture retentions properties as a function of the blend-modified polysaccharide edible film variables. The effect of the WVP was highly significant ($p < 0.05$) for the moisture-retention ability (Table 2), and it was considered the highest coefficient of

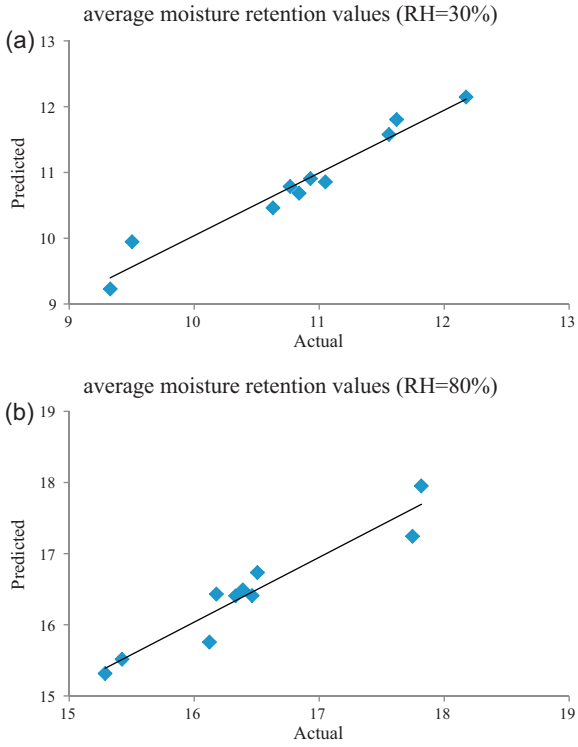

Fig. 2. Predicted and experimental values of (a) the average moisture retention values at RH = 30% and (b) the average moisture retention values at RH = 80% for the blend-modified polysaccharide edible films.

the main linear effects. Similar results have also been reported by other researchers (Bosquez-Molina, Guerrero-Legarreta, & Vernon-Carter, 2003; Lin et al., 2011). These results can be explained by the fact that the water barrier property of the co-blended film most related to film moisture is the retention property (Bosquez-Molina et al., 2003; Lin et al., 2011). The fitted models were suitable, showing significant regression, no lack of fit, and satisfactory coefficients (Table 2).

The predicted values were obtained through a model-fitting technique using the Design Expert software. Fitting the data to a linear model and the subsequent ANOVA showed that the linear model was suitable. There was a suitable relationship between the predicted values and the experimental values (Fig. 2). The model was used to find an optimal region for the response variable studied and to define the relationship between the three independent variables and the response variables. The optimum blending was found to be PUL/ALG/PGA-CO, which gave the best moisture-retention ability.

For validation of the model, the acceptability of the response surface equations was checked through a comparison of the experimental and fitted values predicted by the response regression models. No significant difference ($p > 0.05$) was found between the experimental and predicted values.

3.4. Changes in color and film transparency

Film color is an important index in terms of general appearance and consumer acceptance. The L^*, a^*, and b^* values of the blended films are shown in Table 3. There were no significant differences ($p > 0.05$) in the L^* value between all of the films. In general, the ALG film exhibited a slight yellow appearance that darkened as the thickness increased. The blended films presented increases in a^* and b^* with the direct and co-dried addition of PGA, PEC, CAR, or ALO, indicating that the film became more yellowish after the

Table 3
Thickness, color, and transparency of the blend-modified polysaccharide edible films.[a]

Blending method	Blend-modified polysaccharide edible films	Film thickness (mm)	L^*	a^*	b^*	T
Direct addition	PUL/ALG	45.17 ± 0.47EF	86.06 ± 0.19A	0.42 ± 0.04F	0.77 ± 0.04F	1.62 ± 0.04A
	PUL/ALG/PGA	48.30 ± 1.01D	86.00 ± 0.20A	0.54 ± 0.04E	1.03 ± 0.07E	0.67 ± 0.05EF
	PUL/ALG/PEC	49.03 ± 0.66D	85.96 ± 0.12A	0.63 ± 0.03DE	1.06 ± 0.06E	0.78 ± 0.04CD
	PUL/ALG/CAR	51.43 ± 0.84B	85.92 ± 0.10A	0.71 ± 0.03C	1.09 ± 0.05E	0.83 ± 0.02C
	PUL/ALG/ALO	52.60 ± 1.17AB	85.76 ± 0.03A	0.87 ± 0.05A	1.17 ± 0.06DE	1.02 ± 0.04B
Co-dried addition	PUL/ALG-CO	44.15 ± 1.00FG	86.02 ± 0.11A	0.47 ± 0.04EF	0.80 ± 0.04F	1.57 ± 0.09A
	PUL/ALG/PGA-CO	43.26 ± 0.97G	85.85 ± 0.08A	0.54 ± 0.03E	1.37 ± 0.09C	0.64 ± 0.02F
	PUL/ALG/PEC-CO	49.55 ± 0.30CD	85.99 ± 0.11A	0.67 ± 0.07CD	1.43 ± 0.05AB	0.72 ± 0.05DE
	PUL/ALG/CAR-CO	51.42 ± 1.22BC	86.04 ± 0.17A	0.73 ± 0.03BC	1.54 ± 0.06A	0.75 ± 0.04D
	PUL/ALG/ALO-CO	54.44 ± 0.89A	85.74 ± 0.08A	0.78 ± 0.03AB	1.21 ± 0.02D	0.94 ± 0.07B

[a] The values are the means ± the standard deviation. Different letters in the same column indicate significant differences ($p \leq 0.05$).

incorporation of PGA, PEC, CAR, or ALO. The differences observed in the films' color could be ascribed to different interactions that may have occurred during the blending process and the storage of PUL/ALG and the different polysaccharides (Prodpran, Benjakul, & Artharn, 2007).

The transparency of the PUL/ALG film was low (indicated by the high value of T), which could be linked to the drying process. During drying, the PUL/ALG film-forming solution was easily influenced by the wind in the oven, as indicated by visible ripples on the surface of the PUL/ALG film, which undoubtedly increased the final T value (Wu, Zhong, Li, Shoemaker, & Xia, 2013). There were significant differences in the T values ($p \leq 0.05$) between the blended films, which indicates that the incorporation of PGA, PEC, CAR, or ALO did affect the transparency of the films.

4. Conclusions

In comparison to the common humectants used, ternary co-blended polysaccharide films prepared using the co-drying method can significantly improve the film properties and moisture retention. Three types of directly mixed biopolymers are often not stable. However, the preparation of a co-dried mixture noticeably decreased the incompatibility of both the particle sizes and the chemical properties of the polysaccharides and increased the thermodynamic compatibility (Ohashi et al., 1991; Salvador et al., 2001); therefore, the compatibility of the three phases was greatly improved. This research indicates that ternary co-blended polysaccharide films prepared through the co-drying method can be used as an alternative to traditional humectants and applied for the preservation of IMFs. With the exception of the traditional measuring methods, the assessment of the moisture retention properties of humectants can be performed based on the film formed on the food surface, and this measurement can be used for the total assessment of four film parameters, namely WVP, TS, E%, and OP.

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