EFFECTS OF STORAGE TEMPERATURE AND RELATIVE HUMIDITY ON THE PROPERTIES OF EGG WHITE PROTEIN-BASED FILMS OBTAINED THROUGH EXTRUSION AND CALENDERING PROCESSES

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Packaging—Master of Science

2014

ABSTRACT

EFFECTS OF STORAGE TEMPERATURE AND RELATIVE HUMIDITY ON THE PROPERTIES OF EGG WHITE PROTEIN-BASED FILMS OBTAINED THROUGH EXTRUSION AND CALENDERING PROCESSES

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The goals of this study were: (1) to identify the conditions for egg white protein (EWP) film making through extrusion and calendering processes for industry adaptation, and (2) to investigate the current possibilities of the EWP film as a food packaging material by inquiring into the effect of surrounding environment on its properties and by comparing EWP film with PLA. The necessary processing conditions of extrusion and calendering to obtain EWP films were identified and led to transparent (90-94% at 600nm), continuous, and uniform films with a thickness of 110 μ m. Exposing the films to combinations of temperature (4 or 23°C) and RH (0, 55, or 95%) showed that temperature, RH, and their interactions have significant effect on the properties of EWP films. At 23°C, the film has higher transmittance and lower water and ethanol permeability than at 4°C ($p \leq 0.05$). The decrease in RH increases the flexibility and thickness but it decreases the water permeability and transmittance of the films significantly ($p \leq 0.05$). The films became more reddish and less yellowish at 55%RH. Neither temperature nor RH had an effect on the tensile strength, first second-order transmission temperature, fungal resistance and lightness of the films. Comparison of EWP films with PLA films shows that both materials have similar optical properties but different tensile and barrier properties. The EWP films reduced the growth of *P. pinophilum* and *A. niger* after 48 hours at 100%RH and 23°C. This study proves the feasibility of producing EWP films using the most common filmmaking processing technology and shows that EWP film can be used for specific food packaging applications.

ACKNOWLEDGMENTS

First and foremost, I would like to offer my sincerest gratitude to my major advisor, **Dr. Eva Almenar**, who introduced me to this topic and who has provided me with guidance and support from the start. I attribute the completion of this thesis and my Masters study to her encouragement and effort.

I am also extremely indebted to my committee members; **Dr. Laurent Matuana**, who has always been very generous with his time, and **Dr. Perry Ng** who has always been very attentive throughout the process. I appreciate their understandings and their insightful comments.

I owe a special gratitude to **Dr. Jaime González-Buesa**, who has been an invaluable friend and mentor. His patience, enthusiasms, and immense knowledge enabled me to complete my research successfully.

Extended gratitude is expressed to the students in Dr. Almenar's research group who have shared this journey with me. I would also like to thank fellow graduate students and the faculty/staff at the School of Packaging who have helped me out by offering their knowledge and supports throughout my graduate program.

A thank you is extended to the Michigan State University **School of Packaging** for awarding me a graduate fellowship that helped fund my graduate program.

Finally, I would like to thank my family for their love and support. Thanks to my parents for giving me the opportunity to study in the other side of the world. Thanks to my brother for being my reliable companion, and thanks to my sister for her words of encouragement.

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1. INTRODUCTION

Due to the economic and environmental issues surrounding petroleum-based plastics, there has been an increased pressure in finding more sustainable plastics. This move towards sustainability has stimulated research in the field of bioplastics. Plant and animal proteins are readily available and have been proven to be adequate raw materials for bioplastic development (Hernandez-Izquierdo and Krochta, 2008). Recent studies have shown the potential of egg white protein (EWP) as raw material for the development of a highly transparent bioplastic (Jerez et al., 2007; Lee et al., 2013; Martínez et al., 2013; Félix et al., 2014). These studies have focused on the development of EWP bioplastic by compression molding and injection molding processes.

Despite the importance of the extrusion process, the most common processing method for plastics, limited information is available on the extrusion of EWP. To our knowledge, there is only one study related to EWP extrusion and this investigates the use of extrusion to produce EWP-starch bioplastic (González-Gutiérrez et al., 2011). Therefore, there is no information in the literature about obtaining EWP bioplastic using extrusion. Furthermore, the combination of extrusion with other processing methods to obtain EWP film has not yet been reported. Film making process commonly comprises extrusion and chill roll or calendering. Thus, in this study, the appropriate extrusion and calendering conditions for the development of EWP films were determined. Furthermore, the effect of temperature and relative humidity (RH) on the properties of the developed films was investigated due to the increased attention that bioplastics are receiving as materials for food packaging applications. Most of the published literature regarding protein-based bioplastics focuses primarily on new approaches for their development and not quite as much on exploring the potential of these materials for food packaging applications. The use of this new generation of plastics for such applications requires the understanding of the interactions occurring between food and material as well as of the effect of the surrounding environment. This understanding is needed since these interactions result in changes in the packaging material (e.g. permeability, thermal properties, mechanical properties) and consequently, in changes in the quality and safety of the food product (e.g. flavor, texture, weight loss, microbial growth).

Recognizing the lack of knowledge in the two above-mentioned areas, the goals of this study were: (1) to identify the conditions for EWP film making through extrusion and calendering processes, and (2) to investigate the current possibilities and limitations of EWP films as food packaging materials by inquiring into the effect of both temperature and RH on the thermal, mechanical, barrier (oxygen, water vapor and ethanol) and optical properties as well as on the fungal resistance of the films; and by comparing EWP films to the most commonly used bioplastic in food packaging, poly(lactic acid) (PLA).

2. LITERATURE REVIEW

2.1. Packaging materials for food products

The principal functions of a packaging material intended for use in food packaging is to contain the food, protect it from the environment, and maintain its quality (Coles et al., 2003). Food and beverage plastic packaging contribute 70% of the plastic packaging market in the United States (Comstock et al., 2004). The two categories of plastics used for food packaging purposes are petroleum-based plastic and bioplastic.

2.2. Petroleum-based materials

The plastic packaging materials that have been used in the food industry for the past 50 years are polyethylene or co-polymer based (Cutter, 2006). They are safe, inexpensive, versatile, and flexible (Tice et al., 2003). In 2012, only 9% of all plastic packaging materials were recovered to be recycled, which means the majority of the plastic materials were not reused (Plastics, 2014). The presence of this plastic waste is problematic because once it is dumped in a landfill it will not degrade and will last for an unknown, but certainly long period of time. Rising petroleum costs also generate concern about the efficient production of plastic packaging materials (Weber et al., 2002). Given the increasing rate at which petroleum is being consumed by industry, there are fears that the supplies will dwindle in the future (Khare and Deshmukh, 2006).

2.3. Bio-based materials (bioplastics)

Due to the issues surrounding the petroleum-based plastics and the growing consumer demands for recyclable or natural packaging materials (Koutsimanis et al., 2012), the pressure to find a more sustainable plastic has increased. This move towards sustainability has intensified research in the field of bioplastics. Bioplastics can be categorized based on their production processes and sources (Comstock et al., 2004). One of the categories is polymer extracted from biomass, which includes proteins, lipids, and polysaccharides. This type of raw material has been used as a source of bioplastics for many years (Irissin-Mangata et al., 2001; De Graaf, 2000).

2.4. Protein-based bioplastics

Proteins derived from plants and animals are readily available and are adequate raw materials for bioplastic development (Gennadios, 1996). Various studies have been conducted on the manufacturing of bioplastic based on vegetable proteins (corn, wheat gluten, soy proteins, etc.) and animal proteins (milk proteins, collagen, gelatin, etc.) (Pommet et al., 2003; Cuq et al., 1998). According to Domenek et al. (2004), the biodegradation rate of protein-based bioplastics is one of the highest among fast-degrading polymers. The use of proteins is a promising way to produce biodegradable materials due to their chemical structure. Protein consists of hundreds of amino acids that are able to form weak and strong bonds; therefore, a large range of functional properties of protein-based materials can be obtained (Domenek et al., 2004).

Processing protein-based materials is not an easy task as the softening temperature of protein often lies above their degradation temperature; thus the use of plasticizers is required to increase the range of processing temperatures (Verbeek and Van den Berg, 2010). Two plasticizers that have been widely used to extrude bioplastics; water and glycerol. Water is considered a natural plasticizer of proteins and has been used widely in protein extrusion (Verbeek and Van den Berg, 2010). Glycerol is also an ideal plasticizer due to its small molecular size, which allows it to interpose within the protein chains and modify the forces holding the chain together (Sharmā et al., 2011).

2.5. Egg white protein (EWP)-based bioplastics

Recent studies have shown the potential of EWP as raw material for the development of a highly transparent bioplastic (Gennadios et al., 1996; Jerez et al., 2007; Lee et al., 2013). Researchers have investigated various methods of processing EWP-based bioplastic. The different processing methods are presented below:

2.5.1. Casting

Film formation by casting can be accomplished easily due to the water solubility of EWP. Gennadios (1996) and Ko et al. (2001) developed an egg albumen-based film by mixing egg albumen with glycerol. The solution was then heated to denature the protein and to form the film matrix. After heating, the solution was poured into a container and air-dried in a laminar flow hood.

2.5.2. Compression molding

Lee et al. (2013) successfully prepared egg white protein-based sheets with various water activity and glycerol contents by a thermo-mechanical procedure using compression-molding equipment. González-Gutiérrez et al. (2011) completed similar studies, applying thermo-mechanical procedures to a mixture of EWP and starch. Martinez et al. (2013) focused on compression of EWP sheets containing some antimicrobial compounds.

2.5.3. Injection molding

Félix et al. (2014) successfully obtained albumen/soy protein-based bioplastics through an injection molding process. The authors utilized 100% albumen, 100% soy protein, and mixtures containing varying ratios of the two components.

2.5.4. Extrusion

Despite the practicality of the extrusion process, limited information is available on the extrusion of EWP bioplastics. To our knowledge, the only article on the use of extrusion for producing bioplastic that involves EWP was published by González-Gutiérrez et al. (2011). The researchers created a mixture of albumen, starch, and glycerol, followed by conditioning and extrusion. The authors also developed a procedure that involved a combination of extrusion and compression molding (González-Gutiérrez et al., 2011).

2.6. Potential of EWP Bioplastics as food packaging material

Most of the published articles focused primarily on new approaches in the development of bio-based materials, and not quite as much on the compatibility of the materials to be used in food packaging instances. There is only one study that suggested a potential for EWP bioplastics to be used as food packaging material which demonstrated the antimicrobial activity of compressed EWP bioplastics when mixed with formic acid or oregano essential oil (Martinez et al., 2013). There is a lack of information regarding EWP plastics with regards to its interaction with the storage environment.

2.7. Effect of temperature and relative humidity on protein-based bioplastic properties

The hygroscopic nature of protein-based bioplastic is well known. Thus, plenty of studies have investigated the effects of temperature and relative humidity on the properties of bioplastics. Gontard et al. (1996) studied the effect of temperature and relative humidites on the barrier properties of protein-based film. The authors suggested at low relative humidity (RH) and room condition (25°C), an edible wheat gluten film presents very low oxygen and carbon dioxide permeabilities. The permeability increases exponentially once the RH is increased (Gontard et al., 1996). To our knowledge, the permeability values of EWP bioplastics have not been presented yet. The permeability value is a significant information in exploring the potentiality of EWP bioplastics in packaging a specific food product.

3. CHARACTERIZATION OF EGG WHITE PROTEIN-BASED FILMS OBTAINED THROUGH EXTRUSION AND CALENDERING PROCESSES

3. 1. Materials and Methods

3.1.1. Materials

Spray dried egg white protein (EWP) powder (82.1 ± 0.61% protein, 0.37 ± 0.17% fat, 6.04 ± 0.20% ash, and 3.51 ± 0.22% moisture determined by proximate analysis) was obtained from Rose Acre Inc. (Seymour, IN, USA). Food grade vegetable glycerin (GLY) was obtained from Starwest Botanicals (Sacramento, CA, USA). Magnesium nitrate ($Mg(NO_3)_2$) and potassium sulfate (K_2SO_4) were purchased from Columbus Chemical Industries Inc. (Columbus, WI, USA). Desiccant was obtained from W.A. Hammond Drierite Co. Ltd. (Xenia, OH, USA). Ethanol (purity ≥ 99.5%) was obtained from VWR International (Radnor, PA, USA). Potato dextrose agar was purchased from Sigma-Aldrich Corp. (Saint Louis, MO, USA). Single spore isolates of *Aspergillus niger* and *Penicillium pinophilum* were originally isolated from spoiled food.

3.1.2. Preparation of EWP films

EWP films were obtained by combining extrusion and calendering processes. Extrusion was used to obtain EWP extrudates while calendaring was used to flatten these into films. EWP powder and a mixture of water and glycerol in 2:1:1 ratio were introduced in a co-rotating twin-screw extruder (Model MP19TC2-25, APV Baker; Grand Rapids, MI, United States) with a screw speed of 150 rpm using a gravimetric feeder (Model K2V-T20, K-Tron; Pitman, NJ, United States) and a pump (Model E2 Metripump, Brook Crompton; Hudders Field, England), respectively. The different zones of the extruder were set to various temperature profiles until the desired extrudates were obtained. The resulting extrudates were immediately placed between two Teflon sheets (McMaster-Carr, Elmhurst, IL, United States) and then calendered using a drums drier (Overton Machine Company, Dowagiac, Michigan, United States). Different temperatures and speeds for the drum driers were investigated. The resulting films were peeled off from the Teflon sheets after cooling down for two minutes at room temperature. Material mixture and processing was performed three times to obtain three batches of EWP films (replicates). Figure 1 illustrates the preparation of the EWP films by the use of extrusion and calendering processes.

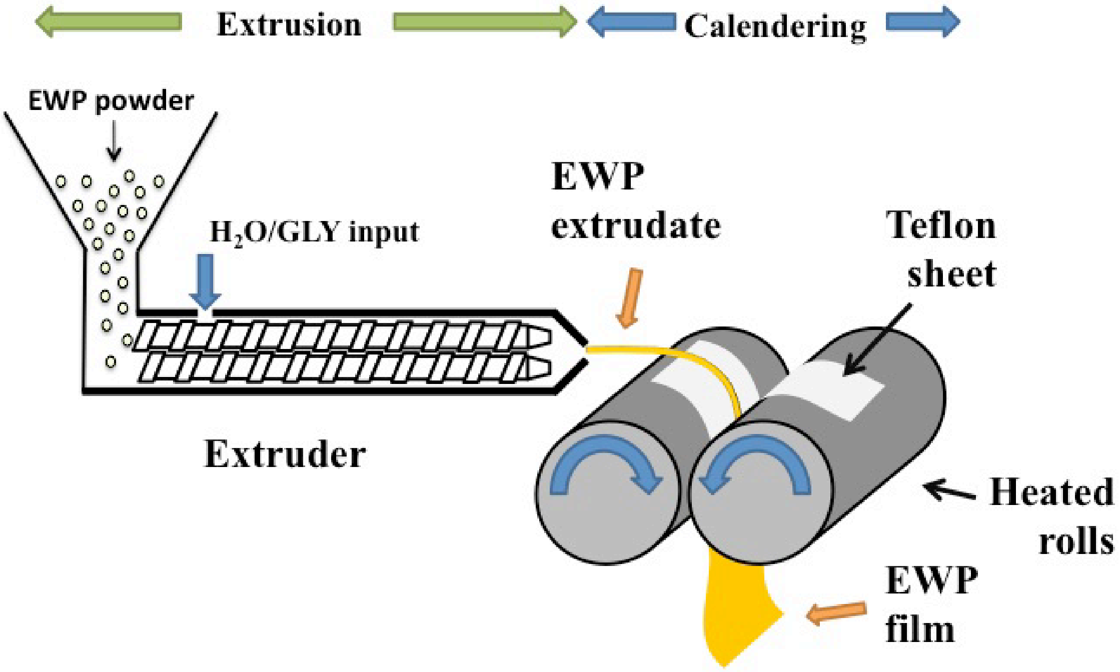

Figure 1: Preparation of EWP films by extrusion and calendering processes (Gonzaléz-Buesa, 2014).

3.1.3. Storage conditions

EWP films were conditioned at six different environments for 48 hours prior to characterization. The different environments (conditioning treatments) resulted from the combination of two different temperatures (4 or 23°C) and three different RH (0, 55 or 95%). A constant temperature and humidity room set at 23°C and a walk-in test chamber set at 4 °C were used to obtain the two temperatures. K_2SO_4 and $Mg(NO_3)_2$ saturated solutions and desiccant were placed inside buckets with air-tight lids to create environments of 95, 55 and 0% RH, respectively, at both temperatures except for 55%RH at 23°C since this was the RH at which the above-mentioned room was set.

3.1.4. EWP film characterization

3.1.4.1. Thickness determination

The thickness of the EWP films after exposure to the different conditioning treatments was obtained by averaging the measurements from five random film positions obtained using an electronic digital micrometer (Fowler® 0-1” Digital Counter Micrometer, Port Washington, NY, USA). The results are expressed in μ m.

3.1.4.2. Thermal characterization

3.1.4.2.1. Differential Scanning Calorimetry

The second-order transition temperature of the EWP films after exposure to the different conditioning treatments was determined by using a differential scanning calorimeter (DSC Q100; TA Instruments, Newcastle, DE) with a liquid nitrogen cooling system. An amount between 6 and 10 mg of each EWP film was hermetically sealed in an aluminum pan (TA Instruments, Newcastle, DE, USA), equilibrated to 0°C, and then heated to 300°C at a rate of 30°C/min under N_2 atmosphere. TA analysis software was used to do the data analysis in accordance with the ASTM Method D3418-12 (ASTM, 2012a). Two samples from each conditioning treatment, each from a different replicate, were analyzed.

3.1.4.2.2. Thermogravimetric analyses

The decomposition temperature of the EWP films after exposure to the different conditioning treatments was determined using a thermogravimetric analyzer (TGA Q50; TA Instruments, Newcastle, DE, USA). An amount between 6 and 10 mg of each EWP film was placed in an aluminum pan (TA Instruments, Newcastle, DE, USA) and then heated from 25 to 600°C at a rate of 30°C/min. The percent weight loss of each sample as a function of temperature under a nitrogen- air (40–60%) atmosphere was analyzed. Two samples from each conditioning treatment, each from a different replicate, were analyzed.

3.1.4.3. Mechanical characterization

EWP films were cut into rectangular strips of 0.1m x 0.01m and then exposed to the different conditioning treatments. The elongation at break (E_b), tensile strength (σ_{max}), and modulus of elasticity (*E*) of each strip were measured according to the ASTM Method D882-12 (ASTM, 2012b) using an Instron Universal Testing Machine UTS SFM – 20 (United Calibration Corporation, Huntington Beach, CA, USA) with a load cell of 453.6kg. A speed of 0.01m/s and an initial grip separation of 0.05m were used. At least six strips from each of the three replicates of each conditioning treatment were evaluated.

3.1.4.4. Barrier characterization

3.1.4.4.1. Water vapor permeability

The water vapor transmission rate (WVTR) of the EWP films after exposure to the different conditioning treatments was measured using a Mocon PERMATRAN® W Model 3/33 Water Permeability Analyzer (Minneapolis, MN, USA) according to the ASTM Method F1249-13 (ASTM, 2013). Film samples were double-masked with sticky aluminum foil, leaving a circular uncovered film area of $0.4x10^{-4}m^2$. Testing for samples stored at 4°C was performed at 10°C which is the lowest temperature the equipment can reach. The samples stored at 23°C were analyzed at that same temperature. RH was matched with that of the storage conditions (55 or 95%). Water vapor permeability (WVP) determination at 0% RH was not carried out since the equipment needs a difference of pressure to measure permeability. The WVP (kg.m/ m^2.s.Pa) of the EWP films was calculated using the following equation:

$$WVP = \frac{WVTR \times L}{\Delta P}$$

where L is the mean film thickness (m) and ΔP is the partial water vapor pressure difference (Pa). The permeability of three samples from each conditioning treatment, each from a different replicate, was measured.

3.1.4.4.2. Oxygen permeability

The oxygen transmission rate (OTR) of the EWP films after exposure to the different conditioning treatments was measured using a Mocon OX-TRAN® Model 2/21 (Minneapolis, MN, United States) according to the ASTM Method D3985-05 (ASTM, 2005). Film samples were double-masked with sticky aluminum foil, leaving a circular uncovered film area of $0.4 \times 10^{-4} m^2$. Testing for samples stored at 4°C was performed at 10°C which is the lowest temperature the equipment can reach. The samples stored at 23°C were analyzed at that same temperature. RH was matched with that of the storage conditions (0, 55, or 95%). Samples stored at 95%RH were tested for permeability at 90%RH because this is the highest RH the equipment sensor can perform. The oxygen permeability (OP) (kg.m/ m^2.s.Pa) of the EWP films was calculated using the following equation:

$$OP = \frac{OTR \times L}{\Delta P}$$

where L is the mean film thickness (m) and ΔP is the partial oxygen pressure difference (Pa). The permeability of three samples from each conditioning treatment, each from a different replicate, was measured.

3.1.4.4.3. Ethanol permeability

The ethanol permeation rate of the EWP films was measured using permeation cells made from poly(methylmethacrylate). The film samples were double-masked with sticky aluminum foil, leaving a circular uncovered film area of $0.3 \times 10^{-3} m^2$. Cells were filled with 1ml of ethanol and then placed inside buckets and moved to the chambers at 4 or 23°C. The buckets were modified by equipping these with inlet-outlet ports that allowed the air from a cylinder to pass through the bucket and thus, to remove from the bucket headspace the ethanol permeated from the cells during storage. Precision needle valves were used to maintain a constant airflow rate of about 200 mL/min. The RH of the bucket headspace was controlled by humidifying the air of the inlet-port using washing bottles containing K_2SO_4 and $Mg(NO_3)_2$ saturated solutions. Washing bottles were not used for the experiments at 0%RH. No buckets were used for the experiment 55%RH-23°C. The weights of the cells were recorded daily using an analytical balance (OHAUS Corp., Parsippany, NJ, USA). Linear regression-derived slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate the ethanol transmission rate (EtOHTR). The ethanol permeability (EtOHP) ($kg.m/m^2.s.Pa$) of the EWP films was calculated using the following equation:

$$EtOHP = \frac{EtOHTR \times L}{\Delta P}$$

where L is the mean film thickness (m) and Δ P is the partial ethanol pressure difference (Pa). The permeability of three samples from each conditioning treatment, each from a different replicate, was measured.

3.1.4.5. Optical characterization

3.1.4.5.1. Color

The color of the EWP films after exposure to the different conditioning treatments was measured using a Labscan XE colorimeter (Hunter Laboratories, Reston, VA, USA) and characterized using the CIE L*a*b* system. The variable L* represents lightness ranging from 0% to 100%. The variable a* ranges from green (negative) to red (positive) and variable b* ranges from blue (negative) to yellow (positive). Five measurements from each replicate from each conditioning treatment were taken.

3.1.4.5.2. Transmittance

The transmittance (%) of each EWP films after exposure to the different conditioning treatments was measured using a spectrophotometer (Lambda 25 UV/VIS Spectrometer; PerkinElmer Instruments, Waltham, MA, USA) equipped with an integrating sphere in the spectral range from 300 to 850 nm and with a scan speed of 480 nm per minute. The transmittance values obtained at a wavelength of 600 nm are reported. Five measurements from each replicate from each conditioning treatment were taken.

3.1.4.6. Fungal resistance

The fungal resistance of the EWP films after exposure to the different conditioning treatments was measured according to a slightly modified ASTM Method G21-13 (ASTM, 2013b). 2 cm x 2 cm pieces of EWP film were placed on potato dextrose agar in plastic petri dishes (9-cm diameter). 100 μ l-inoculum (1 x 10^6 spores/mL) of *Aspergillus niger* and *Penicillium pinophilum* was deposited on the surface of the EWP films and the petri dishes were closed with their lids to avoid external contamination. Culture preparation was performed as described in Almenar et al. (2007). Petri dishes containing conidial suspensions without the films were used as controls. Controls and treatments were incubated at 23°C and monitored daily until fungal growth was observed. Once fungal growth was detected, growth of the cultures in both controls and treatments was evaluated daily by measuring the diameter of the colony on the film and agar surface. Measurement of diameters was made using a conventional ruler. Because of the optical transparency of both the agar and Petri dish, these measurements could be made without problem. One sample from each replicate from each conditioning treatment was evaluated.

3.1.4.7. Statistical analysis

A two-factor completely randomized experimental design was used to study the effects of temperature (4 or 23°C) and RH (0, 55 or 95% RH) on the thickness and the mechanical, barrier, and optical properties of the EWP films. Minitab 16 (Minitab Inc., State College, PA, USA) was used to perform a one-way analysis of variance (ANOVA; Tukey test; $p \leq 0.05$) between the means to determine the effect of temperature and RH on each of the investigated film properties. SPSS 21 (IBM Corporation, Armonk, NY, USA) was used to perform a two-way ANOVA (Tukey test; $p \leq 0.05$) between the means to determine interactions between temperature and RH.

3. 2. Results and discussions

3.2.1. Extrusion and calendering processing conditions for EWP film development

The adequate extrusion and calendering processing conditions to obtain EWP films were determined from trial-and-error experiments. Table 1 summarizes the different combinations of processing conditions used for both extrusion and calendering during the different experiments. Desired EWP extrudates were obtained when the five different temperature zones of the extruder from feeder to die were 40°C - 50°C - 60°C - 70°C - 75°C. Films with desired properties were obtained from the extrudates when the drums drier was heated at 115 – 120°C and its rotational speed was 0.111 rpm. The resulting films were transparent, continuous, and uniform, and had a thickness of 110 ± 10 μ m (Photo 1).

Extrusion	**Calendering**		**Results**
Temperature profile (Extruder feed zone – die) (°C)	Drum dryers temperature (°C)	Rotational speed (rpm)	
50 – 80 – 80 – 100 – 100			Extrudates with hard texture that could not be calendered.
40 – 40 – 40 – 50 – 60			Liquid-like extrudates.
40 – 50 – 65 – 75 – 80			Failed to obtain uniform shaped extrudates due to overheating of extruder's die.
40 – 50 – 60 – 70 – 75	150	0.33	Cloudy EWP films.
40 – 50 – 60 – 70 – 75	150	0.167	Transparent EWP films with breakage in the center of the sheet.
40 – 50 – 60 – 70 – 75	115 - 120	0.111	EWP films with desirable properties.

Table 1: Extrusion and calendering processing conditions used to obtain EWP films.

Figure 2: EWP film obtained by extrusion and calendering processes.

3.2.2. Thickness of EWP films

The thicknesses of EWP films after exposure to different conditioning treatments are presented in Table 2. Temperature did not affect the thickness of the EWP films (P = 0.091; Table 3). In contrast, RH had a significant effect on the thickness of the EWP films (P = 0.008; Table 3). The higher the RH, the lower the film thickness. This phenomenon can be attributed to a loss of glycerol with increasing RH that led to more bonding between polymer chains, thereby reducing free volume. However, the decreased film thickness by increasing RH was not observed for films stored at 23°C-95%RH (Table 2). This was due to an interaction between temperature and RH (P = 0.007; Table 3). More absorption of water by the EWP polymer matrix occurred due to the more surrounding water and the higher temperature. These water molecules increased the free volume of the polymer matrix, thereby increasing thickness. Additionally, the water absorbed by the polymer matrix was high enough to substitute the loss of glycerol observed in films exposed to other storage conditions. Interactions between the temperature and RH were not observed in EWP film stored at RH lower than 95% (Table 2).

Temperature (°C)	RH (%)	Thickness (μm)
4	0	122.3 ± 25.4^{Aa}[1]
	55	105.2 ± 22.7^{ABa}
	95	96.6 ± 19.2^{Bb}
23	0	117.4 ± 12.1^{Aa}
	55	105.9 ± 10.0^{Ba}
	95	120.7 ± 14.4^{Aa}

Table 2: Thickness of EWP films after exposure to different conditioning treatments.

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[1] Different uppercase letters indicate significant differences ($p < 0.05$) caused by RH and different lowercase letters indicate significant differences ($p < 0.05$) caused by temperature.

Factor	Variable								
	Thickness	Tensile			Ethanol Permeability	Color			Transmittance
		Break Elongation (E_b)	Tensile Strength (σ_{max})	Modulus of Elasticity (E)		L	a*	b*	
T	0.091	0.317	0.674	0.055	0.003*	0.266	0.325	0.879	0.001*
RH	0.008*	0.001*	0.024*	0.022*	0.000*	0.387	0.001*	0.001*	0.000*
T*RH	0.007*	0.020*	0.206	0.004*	0.014*	0.263	0.630	0.562	0.023*

Table 3: P values resulting from Univariate ANOVA (SPSS) (*$p < 0.05$ indicates effect of factor (single or combined (interaction)) on each variable)

3.2.3. Thermal properties of EWP films

Fig. 2 presents the DSC thermogram of the EWP films stored at varying temperatures and RH. This thermogram shows two endothermic peaks for the EWP films. Both peaks were associated to second-order transition temperatures. The first second-order transition temperature of all the EWP films occurred at ~ 150°C ($p > 0.05$; Table 4). Similarly, Lee et al. (2013) reported a first second-order transition temperature of around 150°C for compressed EWP sheets. The second second-order transition temperature of all the EWP films occurred between 260-270°C (Table 4). Significant differences ($p \leq 0.05$) were observed between the second second-order transition temperatures of the EWP films conditioned at 23°C. Films stored at 0%RH had a second second-order transition temperature significantly higher ($p \leq 0.05$) than films stored at other RH. This could be attributed to the less exposure to water molecules and consequently, absorption of these, which resulted in a more heat-stable film. This difference was not observed for the EWP films when stored to 23°C most likely due to the reduced absorbance of water due to the lower temperature. It has been suggested that this second endothermic peak corresponds to the decomposition temperature of the EWP (Jones et al., 2013). However, due to the instrument limitation, DSC is unable to show the behavior of the EWP matrix at temperatures above 300°C and therefore, to confirm or deny Jones et al.' claim. In order to study this behavior, TGA was performed on the EWP films as well. Fig. 3a and 3b show the TGA thermograms of EWP films after exposure to the different conditioning treatments. Significant weight loss of EWP films can be observed starting around 190°C up to 600°C which correlates with results obtained by Lee et al. (2013) and Jones et al. (2013) for compressed EWP bioplastics. In Fig. 3a, the first peak could be explained as moisture loss. A similar subsidence was observed for films made from proteins like soy protein isolate and zein in the same temperature range and this was reported to be very likely due to the loss of moisture (Ogale & Cunningham, 2000; Oliviero et al., 2010). The peaks starting around 190°C to 400°C can be explained as the decomposition of glycerol and the breakage of bonds between EWP chains. A complete breakdown or decomposition of EWP films can be seen started around 500°C until around 580°C where the weight percentage of EWP films dropped to 0%. The decomposition temperature of EWP by itself is 136.5°C ± 3°C (Sharma, 2008). Its decomposition temperature shifted to a higher temperature after processing due to absorption of moisture and reorganization in the polymer matrix due to the presence of GLY (Chen et al., 2005).

Higher peaks around 275-300°C are observed for films exposed to 55 and 95% RH at 23°C (Fig. 3a). These peaks indicates that breakage of polymer chains occurred earlier in these samples This phenomenon could be attributed to the films being exposed to more water molecules at higher temperature and consequently resulted in non-bonded water. Non-bonded water has been reported as the reason for the faster decomposition rate of other bio-based polymers like PLA when blended with modified starch (Joo et al., 2011). These results verify that the significant differences shown in Table 4 for the EWP films stored at 23°C were caused by differences in water content.

Temperature (°C)	RH (%)	First second-order transition temperature (°C)	Second second-order transition temperature (°C)
4	0	147.7 ± 5.4^{ns}	261.0 ± 2.3^{Aa1}
	55	143.3 ± 0.5^{ns}	264.9 ± 2.6^{Aa}
	95	139.4 ± 2.5^{ns}	267.7 ± 4.7^{Aa}
23	0	128.2 ± 17^{ns}	265.9 ± 0.1^{Aa}
	55	148.5 ± 3.5^{ns}	260.4 ± 0.8^{Ba}
	95	150.0 ± 3.5^{ns}	259.7 ± 1.9^{Ba}

Table 4: First and second second-order transition temperatures of EWP films after exposure to different conditioning treatments.

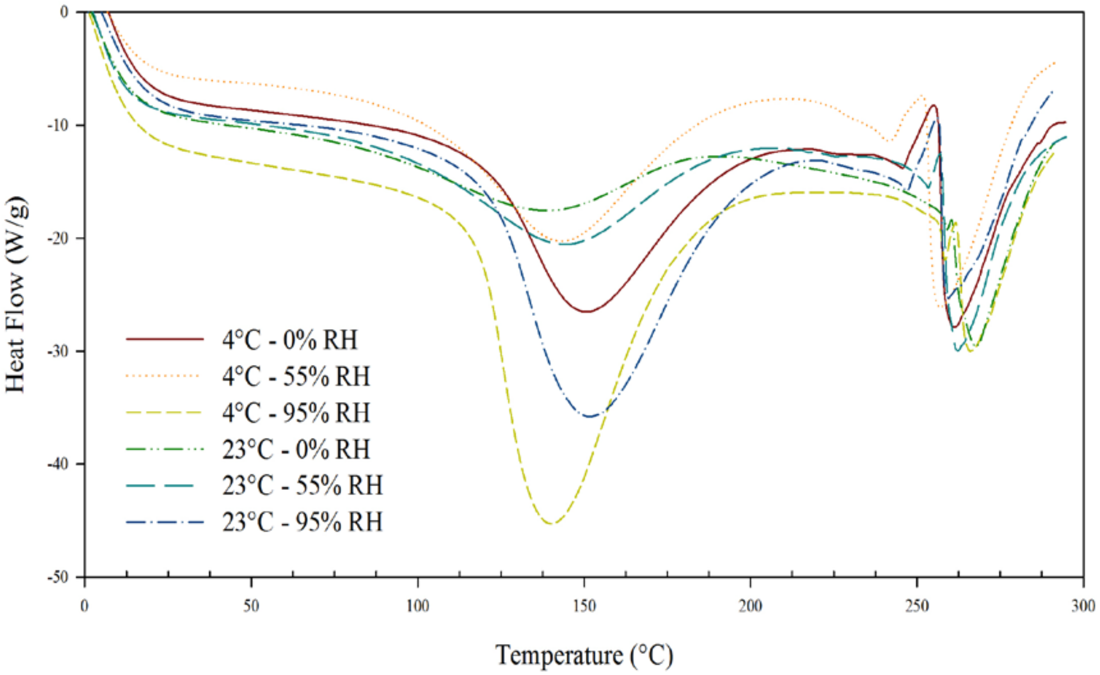

Figure 3: DSC thermograms of EWP films after exposure to different conditioning treatments.

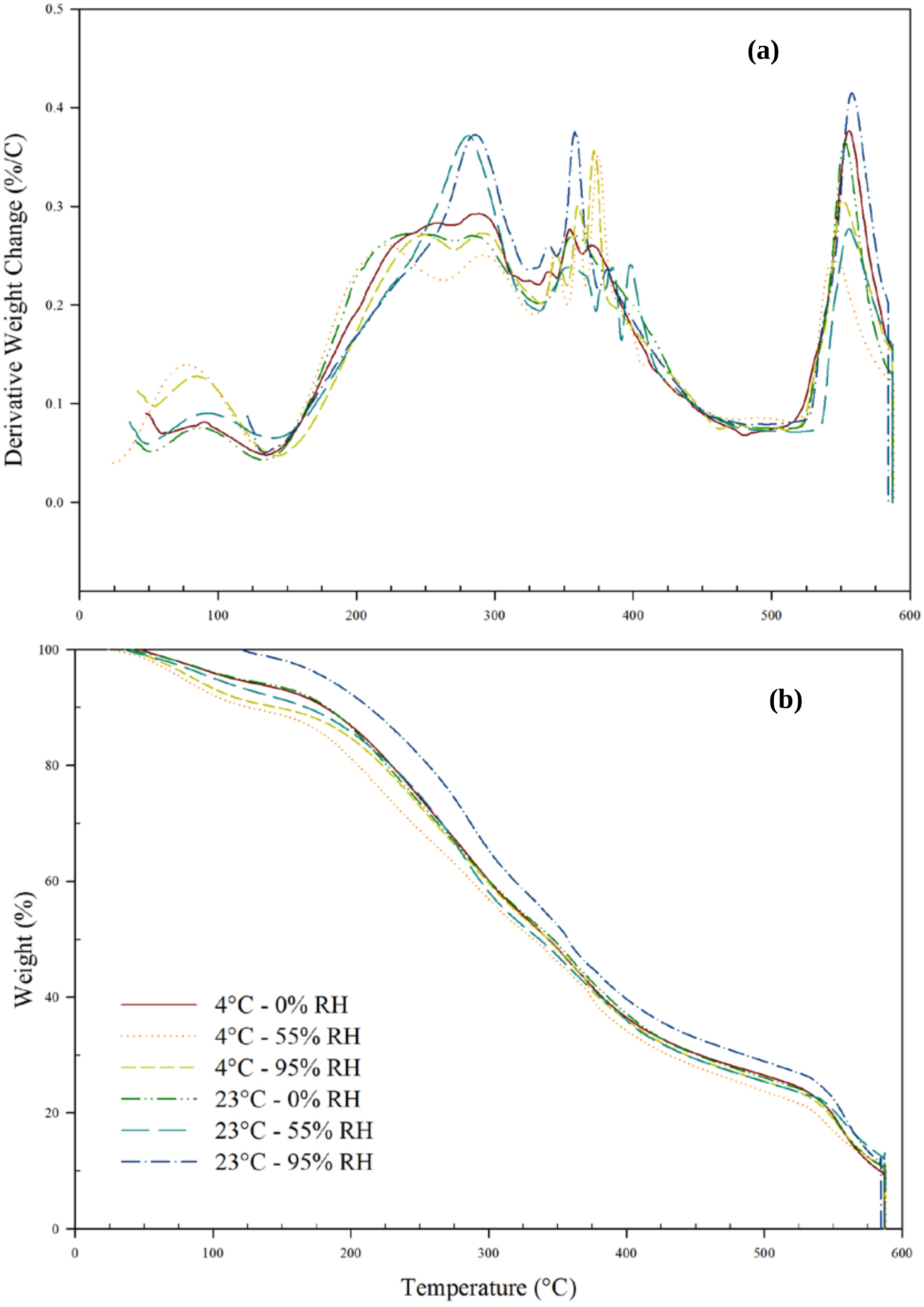

Figure 4: TGA thermograms ((a) derivative weight change and (b) weight loss) of EWP films after exposure to different conditioning treatments.

3.2.4. Mechanical properties of EWP films

The flexibility and stretchability (E_b), the maximum tensile stress sustainable before breakage (σ_{max}), and the rigidity and stiffness (*E*) of EWP films exposed to the different conditioning treatments were measured in the machine direction and the results are presented in Fig. 4. EWP films had an E_b ranging from 17.7% to 36.7%, a σ_{max} between 3.32 MPa and 4.21 MPa, and an *E* between 40.7 MPa and 94.7 MPa. These values are lower for E_b and σ_{max}, and higher for *E* than those reported for compressed EWP bioplastic in the literature (González-Gutiérrez et al, 2010; Lee et al, 2013). The different values can be attributed to the lower GLY content of our films compared to the compressed sheets (25% vs. 35%-45%). The effect of glycerol content on the tensile properties of EWP has been reported previously (Lee et al, 2013). Comparing the mechanical properties between EWP film and PLA film (Joo et al., 2011) at the same storage conditions of 23°C and 55%RH shows that the EWP film is more flexible (36.7, compared to 3.5%), more breakable (3.9, compared to 37.9 MPa) and less rigid (54.5 MPa, compared to 2.4 GPa).

RH had an effect on the mechanical properties of the EWP films while temperature did not (Table 3). RH significantly ($p \leq 0.05$) affected the E_b of the EWP films at both storage temperatures. Independently of the storage temperature, EWP films did not differ in flexibility when stored at 0 or 55%RH. EWP films stored at 95%RH are less flexible ($p \leq 0.05$) than EWP films stored at 55%RH or lower. A significant ($p \leq 0.05$) change of the *E* of the EWP films with RH was observed at both storage temperatures as well. At 4°C, the EWP films were most rigid ($p \leq 0.05$) when stored at 95%RH than at 55%. While at 23°C, the EWP films were more rigid ($p \leq 0.05$) when stored at 0% than at 55%RH or higher. This result suggested that the condition at which the EWP polymer matrix completely absorbed the water molecules occurred when samples exposed to higher RH at lower temperature and lower RH at higher temperature. These conditions resulted in a rigid structure due to less free volume in between the polymer chains. The σ_{max} of the EWP films seems to decrease as the RH increases but the changes were not significant ($p > 0.05$). These results correlate with the results from the one-way ANOVA for storage temperature and RH.

A significant interaction between temperature and RH affected the E_b and E of the EWP films but not their σ_{max} ($P = 0.020$ and 0.004 for E_b and E, respectively; Table 3). Significant differences ($p \leq 0.05$) caused by temperature were only observed in the E_b of the EWP films stored at 55%RH, with films at 23°C being significantly ($p \leq 0.05$) more flexible than those stored at 4°C. Similarly, significant differences ($p \leq 0.05$) caused by temperature were only observed in the E of the EWP films stored at 0%RH, with films stored at 23°C being significantly ($p \leq 0.05$) more rigid than those stored at 4°C. The higher flexibility and rigidity at higher temperatures are attributed by the more water absorbed by the polymer matrix. Water is a plasticizer, which explains the flexibility, and the water molecules filled the voids between the polymer chains resulting in a denser or a more rigid structure.

Based on the result from all different treatments, EWP films show the most appropriate properties for food packaging applications (more flexibility, less breakability, and less rigidity) when stored at 55%RH and 23°C.

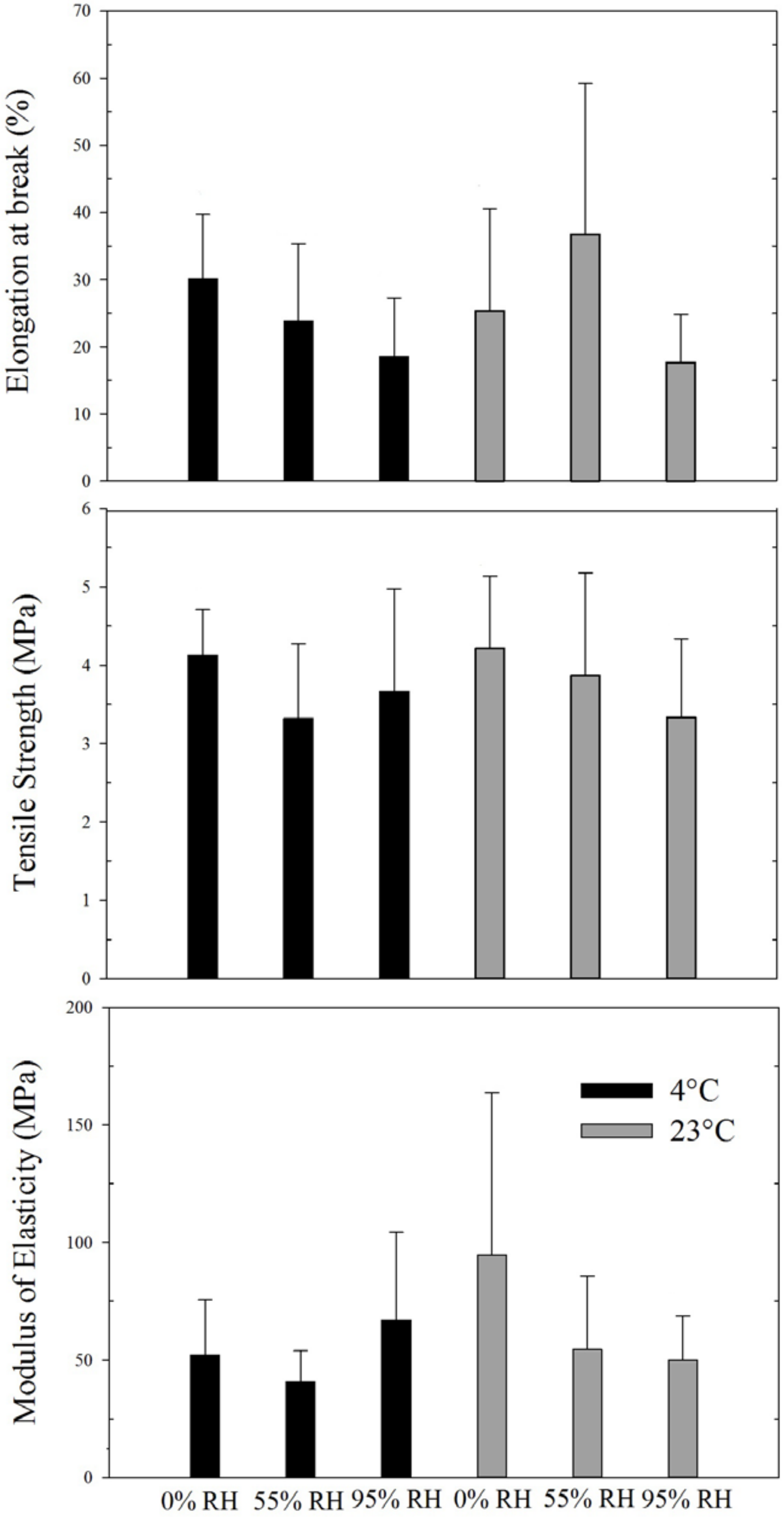

Figure 5: Tensile properties of EWP films after exposure to different conditioning treatments.

3.2.5. Barrier properties of EWP films

The WVP. OP, and EtOhP of the EWP films after exposure to the different conditioning treatments are presented in Table 5. WVP and OP were studied since oxygen and water are two main factors affecting the shelf life of food products. Ethanol permeability was studied as ethanol is a compound typically found in the aroma profile of food products as well as an off-flavor developed by fruits and vegetables under stress caused by low O_2 levels and/or high CO_2 levels.

Both temperature and RH had an effect on the WVP of the EWP films (Table 5). The WVP of the EWP films was higher ($p \leq 0.05$) at 4 °C than at 23°C regardless of RH. The authors do not have an explanation for this contradiction. Permeability of petroleum-based films increases with the rise of temperature following an Arrhenius equation (Hernandez et al., 2000).. At 95%RH, the WVP of the EWP films was higher ($p \leq 0.05$) than at 55%RH regardless of temperature. This higher permeability can be attributed to the more absorption of water by the glycerol in the polymer matrix during storage because of the more available water in the environment. The water molecules increased the free volume of the polymer matrix, thereby increasing permeability. The plasticizing capability of water has widely been studied and reported (Hernandez-Izquierdo & Krochta, 2008; Lee et al., 2013). In addition, the less compact structure of EWP could have allowed the loss of more glycerol, which resulted in higher water diffusion through the films (Miller and Krochta, 1997). The permeability of EWP film at 95%RH and 23°C was 2.3 $x10^{-12}$ kg.m/ m^2.s.Pa and is four orders of magnitude higher than that of commercially available bio-based plastics such as PLA under the same analyses conditions (Almenar & Auras, 2010; Joo et al., 2011; González-Buesa et al., 2014). Therefore, the EWP film is not a good choice to package water-sensitive food but it is a good choice for allowing water to scape from the package avoiding condensation issues in packaged non water-sensitive food products. Both temperature and RH also had a significant effect on the OP of the EWP films. Significant differences in OP resulting from temperature and RH were observed in the EWP films stored at higher RH. The increase of OP as the temperature increases was expected, which correlates with Arrhenius equation. The increase of OP as the RH increases was due to the plasticizing effect of water as mentioned previously. The OP of the EWP film at 0%RH and 23°C was 0.26×10^{-18} kg.m/ m^2.s.Pa, which is in the same range as that of the PLA film under the same conditions (5.67×10^{-18} kg.m/ m^2.s.Pa) (González-Buesa et al., 2014). This finding suggested that the EWP film would work as well as the PLA film for food packaging applications where protection to avoid oxidation is needed.

Temperature (°C)	RH (%)	Permeability (kg.m/ m^2.s.Pa)		
		Water (x10^{-12})	O_2 (x10^{-18})	Ethanol (x10^{-17})
4	0	N/A	1.43 ± 0.73^{Ba}	7.97 ± 2.84^{Aa}
	55	0.26 ± 0.07^{Bb}	15.3 ± 3.58^{Aa}	$14.\ 4 \pm 3.96^{Aa}$
	95	3.32 ± 1.25^{Ab}	4.24 ± 0.86^{Bb}	12.6 ± 1.04^{Aa}
23	0	N/A	$0.26 \pm 1.2E\text{-}2^{Ba}$	2.83 ± 0.84^{Bb}
	55	$0.01 \pm 3E\text{-}3^{Ba}$	2.88 ± 0.66^{Bb}	5.79 ± 0.23^{ABb}
	95	2.32 ± 1.05^{Aa}	40.2 ± 6.24^{Aa}	11.9 ± 4.53^{Aa}

Table 5: EWP films permeability values for water, oxygen, and ethanol after exposure to different conditioning treatments (N/A means “not applicable”) .

The EtOhP of the EWP films was 2.83-14.4 x10^{-17} kg.m/ m^2.s.Pa. This value is in the same range as that of LDPE (3.2×10^{-17} kg.m/ m^2.s.Pa (Robertson, 2013)) and a little bit higher than that of PLA (1.0×10^{-17} kg.m/ m^2.s.Pa (González-Buesa et al., 2014)). There was an effect of both temperature and RH on EtOhP (Table 3). The lower the temperature the higher the EtOhP. As previously mentioned, temperature affects permeability of plastics following an Arrhenius equation and therefore, the authors do not have an explanation for this contradiction. An interaction between the temperature and RH was observed on the EWP EtOHP (P=0.014; Table 3), with the EWP films stored at 23°C increasing in EtOHP with the increase of RH but not the EWP films stored at 4°C.

3.2.6. Optical properties of EWP films

The color of the EWP films after exposure to the different conditioning treatments is presented in Table 5. The color (a* and b*) of the EWP films was affected ($p \leq 0.05$) by RH, but not by temperature (Table 3). The EWP films become more reddish and less yellowish at 55%RH than at either lower or higher RH for both temperatures. The authors have no explanation for such changes. The lightness (L*) of the EWP films was not affected by neither the temperature nor the RH (Table 3). When comparing our results to published information pertaining L*, a* and b* of compressed EWP bioplastic (Lee et al., 2013), the EWP films are lighter (91.5%, compared to 87%), more reddish (-1.45, compared to -2.4), and less yellowish (2.7, compared to 17) than compressed EWP bioplastic. The difference in color is most likely due to the different thicknesses between our EWP films and the compressed EWP bioplastics. Gennadios and Ghorpade (1996) indicated that the color of protein-based films is affected by thickness. The authors reported that thicker soy-based films were more yellowish than thinner ones. Lee et al. (2013) reported that the thinner the compressed EWP sheet is the lighter and the less green and yellow it becomes. Comparing the color of EWP films with that of other bioplastic with similar thickness like PLA that was stored under the same conditions (23°C and 55%RH; Joo et al., 2011) shows that both bio-based films have similar lightness (91.6 vs. 92.5) and b* values (2.1 vs. 1.6) but not a* values (-1.3 vs. -0.11). Therefore, the EWP film is a little bit more greenish than the PLA film. This should not be a problem from the consumer point of view since there are several commercially available plastics with a green color tinge that are used for food packaging applications like PVC and PVDC.

Temperature (°C)	RH (%)	Color			Transmittance (%)
		L*	a*	b*	
4	0	91.7 ± 0.3^{Aa1}	-1.46 ± 0.14^{ABa}	2.57 ± 0.61^{ABa}	90.2 ± 4.4^{Ba}
	55	91.9 ± 0.4^{Aa}	-1.35 ± 0.12^{Aa}	2.13 ± 0.60^{Ba}	94.4 ± 0.9^{Aa}
	95	91.8 ± 0.3^{Aa}	-1.52 ± 0.16^{Ba}	3.06 ± 0.91^{Aa}	93.3 ± 1.7^{Aa}
23	0	91.6 ± 0.6^{Aa}	-1.47 ± 0.21^{Ba}	2.76 ± 0.99^{Aa}	93.4 ± 0.9^{Bb}
	55	91.6 ± 0.5^{Aa}	-1.30 ± 0.13^{Aa}	2.11 ± 0.63^{Aa}	94.3 ± 0.9^{ABa}
	95	91.8 ± 0.3^{Aa}	-1.45 ± 0.21^{ABa}	2.74 ± 0.75^{Aa}	94.9 ± 0.8^{Ab}

Table 6: Optical properties (color and transmittance) of EWP films after exposure to different conditioning treatments.

The transmittance (%) of the EWP films after exposure to the different conditioning treatments is shown in Table 2. EWP films obtained by extrusion and calendering processes are highly transparent (90-94% at 600nm wavelength). They were more transparent than the compressed EWP bioplastics obtained by González-Gutiérrez et al. (2011) and Lee et al. (2013), which had a transmittance of 38% and 89%, respectively. The improvement of EWP bioplastic transparency could be attributed to the higher orientation of the polymer chains caused by the extrusion and calendering processes. A difference in thickness could be also the reason for the different transparency of the materials. XTemperature significantly affects ($p \leq 0.05$) the transmittance of EWP films at 0 and 95%RH ($P = 0.001$; Table 3). The average transmittance of films stored at 23°C is higher than the average transmittance of films stored at 4°C, specifically for EWP films stored at 0%RH (interaction temperature and RH; $P = 0.023$; Table 3). This may be due to the more water absorbed by the EWP matrix at 23°C than a 4°C, loosening the EWP matrix more and consequently, allowing more light to go through. The decrease in RH at both temperatures decreases the transmittance of the EWP films significantly ($p \leq 0.05$) (P = 0.000; Table 3). The loss of water may result in a tighter polymer matrix that allows less light to transmit through the film.

3.2.7. Fungal resistance of EWP films

The EWP film did not pass the ASTM fungal resistance testing since the film did not avoid the growth of *P. pinophilum* and *A. niger* for 21 days (Fig. 5). However, it needs to be taken into consideration the ASTM method G21 was developed for petroleum-based plastics, which do not interact with the agar of the petri dish as the EWP film does. This interaction most likely resulted in the migration of agar nutrients from the agar into the EWP film, which most likely fed the fungi. In addition, the testing was performed at 100%RH and 23°C instead of 85%RH and 23°C which substantially increases fungal growth. The closing of the petri dishes with their lids created the higher RH environment. This was done to allow the manipulation of the petri dishes to measure fungal colony size and compare controls and treatments. Controls were petri dishes that were filled with the agar and then exposed to inoculated solutions, without EWP films placed in them.

The growth of *P. pinophilum* and *A. niger* during 48 hours at 100%RH and 23°C was less on the films than on the agar. The average growth area of *P. pinophilum* on the film was 1.06×10^{-4} m^2 whereas the average growth area of *P. pinophilum* on the agar (controls) was $2.14 \times 10^{-4} m^2$. Similar results were observed for *A. niger* which had a growth area of 1.01×10^{-4} m^2 on the films and 1.86×10^{-4} m^2 on the agar. Comparing the different treatments, neither temperature nor RH had an effect on fungal growth. This can be attributed to the interaction of the films with the agar, which changed the properties of the films and made this similar overtime.

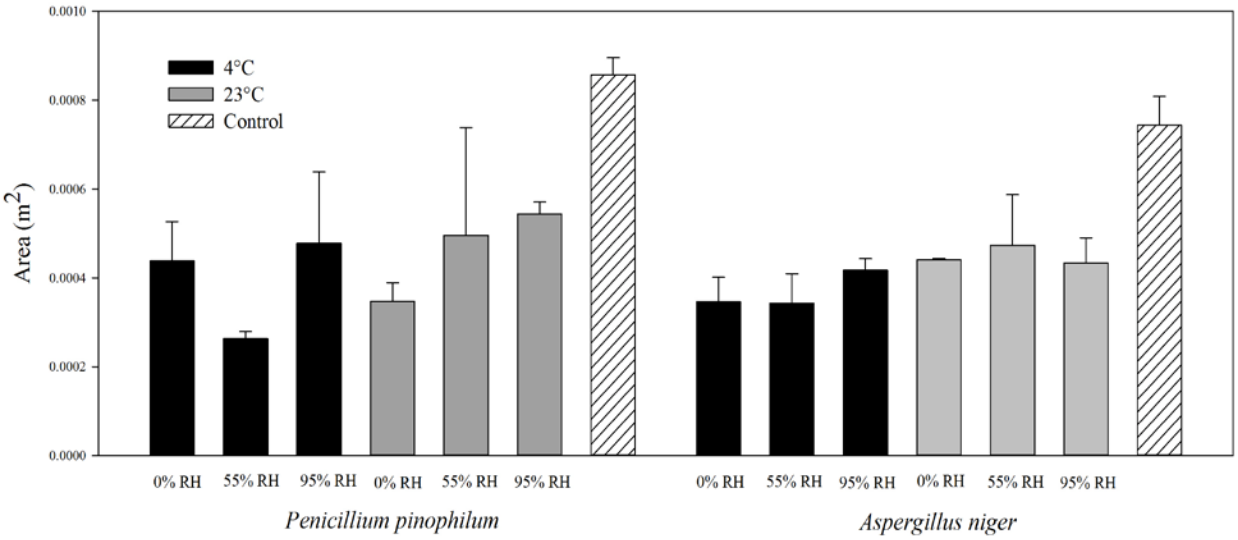

Figure 6: The growth area of *P. pinophilum* and *A. niger* on EWP films after 48 hours incubation.

4. CONCLUSIONS

The processing conditions of both, extrusion and calendering necessary to obtain EWP films were successfully identified and led to transparent (90-94% at 600nm), continuous, and uniform films with a thickness of 110 ± 10 μm. The exposure of the EWP films to combinations of temperature (4 or 23°C) and RH (0, 55, or 95%) showed that both temperature and RH, and their interactions have a significant ($p \leq 0.05$) effect on the properties of EWP films, with the later being greater. At 23°C, EWP film has higher ($p \leq 0.05$) transmittance and lower ($p \leq 0.05$) WVP and EtOHP than at 4°C. The decrease in RH at both temperatures decreases the transmittance and water permeability, and increases the flexibility and thickness of the EWP films significantly ($p \leq 0.05$). EWP films become more reddish and less yellowish at 55%RH at both temperatures. Neither temperature nor RH had an effect on the tensile strength, first second-order transmission temperatures, fungal resistance, and lightness of the EWP films. Interactions between temperature and RH that affected the thickness, elongation at break, modulus of elasticity, EtOHP and transmittance of the EWP films were found. Comparing EWP film to PLA film, the bioplastic most widely used for food applications, at the same storage conditions (0%RH and 23°C) shows that both materials have similar color but different barrier and mechanical properties. The EWP film is more flexible, more breakable, less rigid and more permeable to water and ethanol than the PLA film. The oxygen permeability of EWP is similar to that of the PLA. The EWP films did not avoid the growth of *P. pinophilum* and *A. niger* but did reduce their growth after 48 hours at 100%RH and 23°C. This study proves the feasibility of producing EWP films using the most common filmmaking processing technology and shows that EWP film can be used for specific food applications.

5. FUTURE WORK

The results of this study indicated that the EWP films can be produced using the most common filmmaking processing technology and can be used for specific food applications. EWP films did not pass the ASTM fungal resistance testing, but shows reduction of fungal growth after 48 hours. Therefore, future work would include:

1) Fungal resistance testing at lower temperature (4°C).
2) Develop an active packaging by adding an antimicrobial compound to EWP films.
3) Study the compatibility of EWP films for food packaging application by forming the film into a pouch and then place food items inside the pouch.
4) Investigate the effect of food/package interaction to the properties of the films and the food item itself.

APPENDIX

APPENDIX

DEVELOPMENT OF EXTRUSION METHOD OF EGG WHITE PROTEIN-BASED FILM.

The final extrusion method that led to desired EWP films resulted from several trial-and-error experiments that are compiled in the methods presented below. In all experiments, prior to each extrusion, calibration curves were performed for both the feeder and the pump in order to determine the quantity of the solid and liquid feed per minute necessary to obtain extrudates with stable properties. Calibration curves were obtained by measuring the weight of the EWP powder or the liquid solution per minute for different feed rates and water pump percentages, respectively, and then plotting weight/time (g/min) versus feed rate (rpm) or percentage (%). The feed rates were 50, 100, and 150 rpm. The water pump percentages were 5, 10, and 15%. These settings were selected as the desirable quantity of the liquid and solid feed were within the range of the settings. Measurement of weight was completed twice per setting, and the results were then averaged. However, if the weight varied in one setting, measurement of weight was continued until two or more measurements close in value were obtained. The ratio of the water, glycerol, and EWP used in all extrusions was 1:1:2. This mixture was selected because it was the suitable mixture to extrude stable EWP bioplastic without diffusion of the glycerol that resulted in oily surface of the extrudates (results not shown).

1. Method I

The first method was completed using two extruders, as seen in Fig. 6. Extrudates were obtained using an APV Baker extruder (Model MP19TC2-25, APV Baker; Grand Rapids, MI, United States) with temperature settings of 45 - 100 - 140 - 140 - 100°C from feeder to die, screw speed of 125 rpm and a 4-mm circular die attached at its end. This extrusion settings and the aforementioned raw material mixture created extrudates with glossy appearance (Fig. 6). These extrudates were posteriorly pelletized into small pieces using scissors and then introduced to the second extruder, a microtruder, to obtain EWP sheets. The microtruder was equipped with a single manifold lip die (Randcastle Extrusion Systems, Inc., Cedar Grove, NJ, USA), had its five heating zones set at 80 – 110 – 120 – 145 – 135°C and a screw speed of 500 rpm.

Several parameters were changed in order to determine the best setting to successfully reshaped the EWP extrudate into sheets. Temperature zones were set lower (55 – 55 – 65 – 80 – 100°C) and screw speed was changed between 300 – 700 rpm. After several unsuccessful trials with the extrudates ended up blocking the microtruder, it is understood that the extrudates did not perform well when reprocessed using the microtruder. The microtruder's hopper has a very small opening that allows small quantity of extrudates to go through the mictrotruder. When the extrudates were heated, the glycerol tend to migrate to the surface. The glycerol oiliness did not work well with the vertical design of the microtruder and resulted in extrudates pieces sticking to the wall of the screws instead of moving towards the die. Due to the lack of flow of extrudates, the microtruder could not generate enough shear force and pressure to maintain the outflow of the extrudates to reach the die even when the screw speed was set at a higher rate. In addition, the extrudates had to be slowly fed to the microtruder and this led to longer exposure time for the extrudates that were already inside the microtruder. This longer lead-time resulted in burnt and hardened extrudates that which blocked the flow inside the microtruder. It can be concluded that in order to process EWP bioplastic, a one-time heating process is required. Thus, multiple extrusions are not suitable for EWP film development.

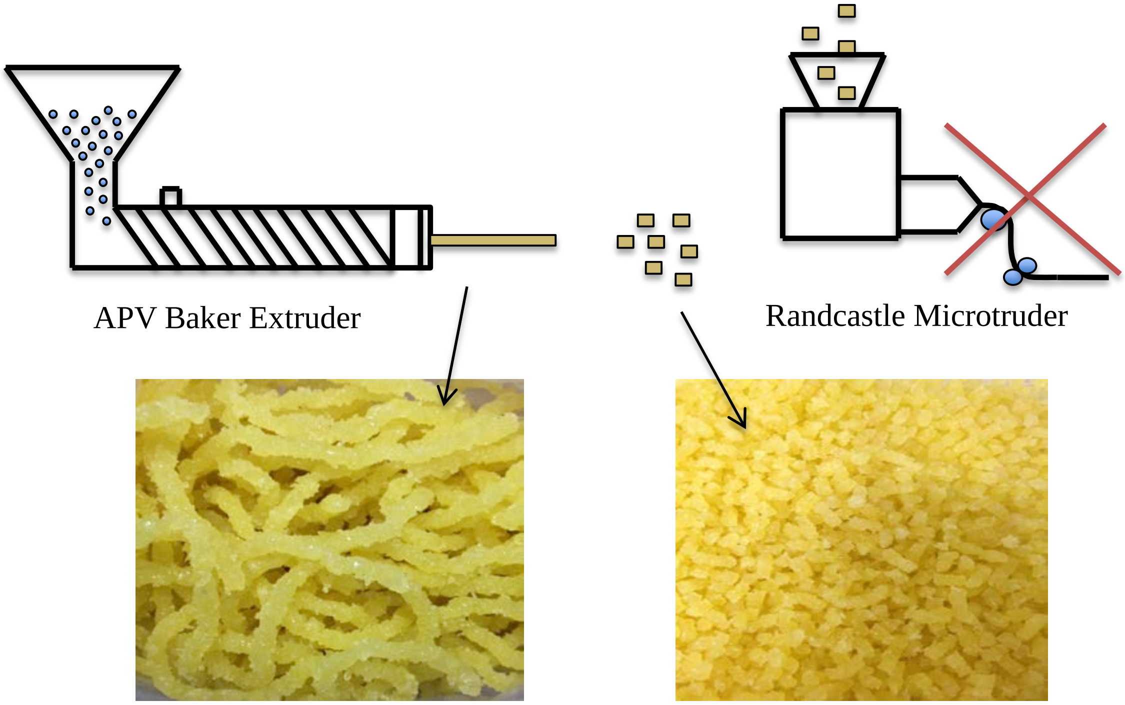

Figure 7: Method I of EWP bioplastic Extrusion

2. Method II

Method II combined the APV Baker extruder and the microtruder’s lip die (Fig. 7). The single manifold flex lip die of the microtruder was attached to the end of the APV Baker extruder through a custom made adapter. In the end, a total of seven heating zones were created by this attachment; five heating zones of the APV Baker extruder, and two extra heating zones for the Randcastle lip die.

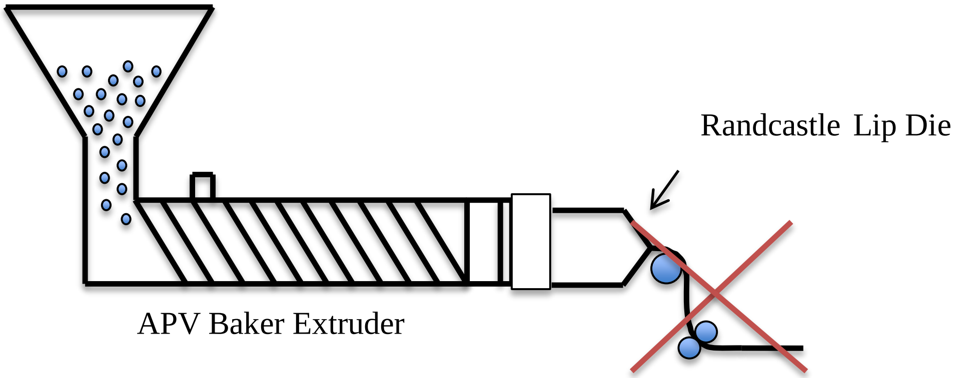

Figure 8: Method II of EWP bioplastic Extrusion

The temperature setting used was 30 – 50 – 90 – 100 – 120 – 140 – 140°C with a screw speed of 200 rpm. Several paremeter changes were completed to determine the best setting to obtain EWP films. Temperature settings were changed into higher and lower temperature and the screw speed was increased due to the extra length, however, none of the settings worked. It was observed that the size of the lip die was too big. It took a long period of time to completely fill the lip die with the EWP mixture. The long period of time to fill the lip die resulted in burnt and hard material that stopped the flow inside the extruder. When the temperatures in all zones were decreased to 60 or 70°C, the material that came out of the lip die has a liquid appearance.

It can be concluded that the lip die from the microtruder was not suitable for extrusion of EWP films. A better chance of obtaining EWP films could be achieved by utilizing a smaller lip die that will not take a long time to fill. However, we cannot ensure that. Additionally, there is no equipment that matched with the specifications that were needed. A further change of method was proposed which resulted in a successful method that is described as method III.

3. Method III

In Method III, extrusion and calendering processes were combined. Calendering was used to flatten the extrudates that were obtained in rod shapes from the APV Baker extruder. The same temperature setting and screw speed as described in Method I were used in order to obtain the rod shaped extrudates. Common calendering process does not reach temperature as high as 140°C. However, a drums drier works in a similar manner to calendering process and can reach to a temperature as high as 160°C (temperature necessary to flatten the EWP extrudates). After the extrudates were obtained, they were placed in between the two drums. Instead of flattening the extrudates, the high heat and rotation broke the extrudates into smaller pieces. The next step following this finding was to reduce the temperature setting used to produce EWP extrudates in order to obtain extrudates with the right texture. Moreover, the speed of the drums is adjustable through manual rotation which allow the extrudates to be exposed to a high temperature only for a short period of time, which avoid burning and color change of the extrudates.

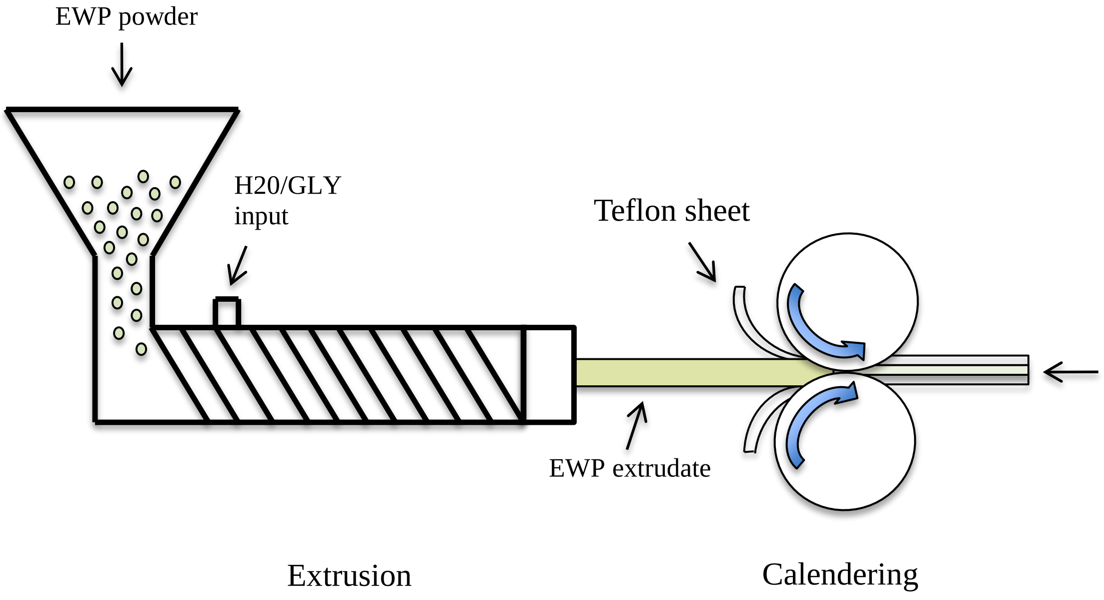

Figure 9: Method III of EWP bioplastic Extrusion

Several temperature combinations were tested (see table I) until obtaining the temperature combination of 40 – 50 – 60 – 70 – 75°C which resulted in extrudates with desired properties to be calendered afterwards. The extrudates solidified but they were still softer in texture compared to the extrudates from method I. Once the extrudates were obtained, they were placed in between two Teflon sheets in order to create easily peelable surface. The drums were rotated at a speed of 0.111 rpm. Once the extrudates and the Teflon sheets completely went through the drums drier, they were cooled down at room temperature for several minutes before being peeled off from the Teflon sheets. This method successfully created a EWP film with flexibility, uniform thickness, and high transparency.

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