EFFECT OF GLYCEROL AND WATER ACTIVITY ON THE PROCESSING AND
PROPERTIES OF EGG WHITE-BASED BIOPLASTICS

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ABSTRACT

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Protein-based bioplastics are a new generation of plastics and are suitable to be produced commercially on a large scale using the same manufacturing methods as petroleum-based plastics. However, proteins require thermoplasticization prior to undergoing these thermal processes. In a first study, the effects of plasticizer type (water and glycerol (GLY)) and amount (0.34, 0.48 and 0.64 A_w and 0, 35, 40 and 45% GLY) on the thermoplasticization of the egg white protein (EWP) were investigated using thermal studies, gravimetric analysis, and morphological studies. The results showed that the combination of 35% GLY content and 0.48 A_w yields an EWP network with a moisture content of about 12% and that this is sufficient to lower its second second-order transition temperature below 170°C, the temperature that marks the onset of thermal degradation of the EWP network. Under these conditions the EWP network can be thermoplasticized at 150°C with less than 15% degradation. The degradation can be reduced further by increasing the GLY content. In a second study, the EWP were compressed to develop egg white-based bioplastics and the effects of GLY (35, 40 and 45%) and A_w (0.34 and 0.48) on the physical, mechanical and morphological properties of compressed egg white-based bioplastics are investigated. Lighter, more reddish and less yellowish sheets with a decreased thickness and transition temperature, improved mechanical properties and constant moisture content can be obtained by increasing A_w. Increasing the GLY content results in the same type of changes but to a lesser extent and increased moisture content. Increasing both, water activity and GLY content, leads to a more pronounced effect in some of these properties.

To Yim Fong, Chen Guan and Hui Qing

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KEY TO ABBREVIATIONS

AFM	Atomic Force Microscopy
CSER	Corporate social and environmental responsibility
DEG	Diethylene Glycol
DMA	Dynamic Mechanical Analysis
DSC	Differential Scanning Calorimetry
E_b	Elongation at break
EG	Ethylene Glycol
EWP	Egg White Protein
Tg	Glass transition temperature
GLY	Glycerol
E	Modulus of elasticity
PEG	Polyethylene Glycol
PG	Propylene Glycol
PHA	Polyhydroxyalkanoate
RH	Relative Humidity
SEM	Scanning Electron Microscopy
SFPI	Sunflower Protein Isolate
S-H	Sulfur-Hydrogen
SPI	Soy Protein Isolate
G'	Storage modulus
S-S	Sulfur-Sulfur

tan δ	Tan Delta
Tc	Temperature of crystallinity
Tm	Temperature of melting
σ_{max}	Tensile strength
TGA	Thermogravimetric analysis
VP-SEM	Variable Pressure Scanning Electron Microscopy
WG	Wheat Gluten
WPI	Whey Protein Isolate

CHAPTER 1

INTRODUCTION

1.1 Motivation

Today the food packaging field is predominated by plastics derived from petrochemical sources like polyolefins, polystyrene, and polyethylene terephthalate. These plastics are relatively inexpensive due to economy of scale and low processing costs. In addition, they present desired physical and mechanical properties, and favorable cost-performance. However, they are not biodegradable and/or compostable and hence, result in waste disposal problems and environmental concerns (Luecha et. al., 2010; Salerno et. al., 2007). In 2010, approximately one-third of the total U.S. municipal solid waste was containers and packaging, which almost half of them made from plastic (Environmental Protection Agency, 2010). Government, industry, and consumers are more and more interested in ways to reduce the municipal solid waste resulting from plastics and to decrease the dependence of the production of plastics on petrochemical sources (Kim 2008; Koutsimanis et al., 2012; Mohanty and Misra 2002). These desires have led to the intensification of research to develop bioplastics for technical applications in recent years (Cuq et al., 1998; Oliviero et. al., 2010). Bioplastics are a new generation of plastics. They are biodegradable plastics made from renewable resources (Stevens 2002) and present advantages like being made from renewable feedstock and biodegradability and compostability which have contributed to the major growth of these plastics in packaging and other fields. Bioplastics are currently a viable alternative to petroleum-based plastics with polylactic acid being the most used among all commercially available bioplastics. However, in general, bioplastics made of biopolymers from renewable feedstock have not yet found wide use in food packaging. Reasons behind this are that such biopolymers present problems when processed with traditional technologies as well as show inferior performances in terms of functional and structural properties (Mensitieri et. al., 2011). However, base formulations are widely being studied to

improve their performance and processing (Hernandez-Izquierdo and Krochta, 2008a; Siracusa et al., 2008; Verbeek and van den Berg, 2011). Among these resources, proteins offer great potential for the production of bioplastics (Gonzalez-Gutierrez et al., 2010; Guerrero et al., 2011) as they are comprised of up to several thousands of amino acids with the capability of forming both weak and strong linkages that result in a stable three-dimensional network stabilized by low-energy interactions and strengthened by covalent bonds (Pommet et al., 2003). Proteins, such as soy protein, wheat gluten, corn zein, and egg albumen that are produced on an annual kiloton scale, have been investigated (Gomez-Martinez et al., 2009; Gonzalez-Gutierrez et al., 2010; Kim, 2008; Tummala et al., 2006). Recent studies have been done on shaping proteins through thermal processing methods, which may lead to compression molding and extrusion and allow production of these bioplastics commercially on a large scale that will save on the economy of costs (Cunningham & Ogale, 2000; Gonzalez-Gutierrez et al., 2011; Sothornvit et al. 2007; Zhang et al. 2001).

In this literature review, knowledge and available research about materials, processing techniques, processing mechanisms and film and mixture (proteins and plasticizers) properties related to thermoplastic protein processing have been discussed and reviewed. Processing techniques such as compression molding and the effect of plasticizers on the different properties of compressed sheets for food packaging applications are of particular interest. The basic materials required for the compression of proteins ("dry process") into films/sheets include a raw material in powder form, which is high in protein contents, and one or more plasticizers. The protein thermoplastification typically involves a combination of water, which will be present in the proteins due to influence from atmosphere exposure, along with one plasticizer that should remain in the film during and after formation of the films or sheets. In literature, compression

molding had been successfully applied to many proteins such as whey, corn, soy and wheat gluten proteins, and the option of extrusion is further explored. Current research on egg white proteins has mainly been done on egg white films formed by the "wet process" or solution-casting method. To date, only a few studies have focused on egg white-based bioplastics. These studies are limited to one glycerol and water activity content and therefore, there is no systematic information about the effects of glycerol and water activity on the properties of egg white-based bioplastics in the literature. Therefore, it is very intriguing to investigate further in the area of "dry process" such as compression and extrusion techniques. It is also important to understand the effects of the amounts of glycerol and water activity on the egg white protein thermoplastification for the optimal compounding of egg white proteins as a precursor step for the manufacture of egg white-based bioplastics using industrial processes like compression.

The study and understanding of plasticizer effect on egg white protein thermoplastic processing will provide us with some knowledge to better control and improve its processing. This is a further step for the commercialization of egg white bioplastics and future applications of egg whites.

1.2 Dissertation Hypotheses and Objectives

General Objective

To investigate the effects of glycerol and water activity on the processing and properties of egg white-based bioplastics

Primary Objectives

1. Understand the effects of glycerol and water activity on the thermoplastification of egg white proteins.

2. Investigate the role of both, glycerol and water activity contents, and of their interaction, on the physical, mechanical and morphological properties of compressed egg-white-based bioplastics.

3. Compare the properties of compressed egg white-based bioplastics with other compressed protein-based bioplastics through the information available in the literature.

Secondary objectives

4. Serve as a foundation for future studies involving other thermal processing of the egg white protein such as extrusion.

5. Improve technology for egg white protein processing.

6. New uses for egg white protein.

Hypothesis

1. Thermal transitions of egg white protein-glycerol-water mixtures will depend on plasticizer type and amount.

2. Thermal transitions of egg white protein-glycerol-water mixtures obtained using differential scanning calorimetry (DSC) can guide optimal compounding as well as temperature selection for adequate compression for egg white proteins into sheets/films.

3. The compounding of egg white proteins with different water activity and glycerol contents will affect the properties of compressed egg white-based bioplastics.

Eventual Outcomes

This project will explore the opportunity for large scale production of egg albumen to reduce the processing cost due to economy of scale and to make it a viable alternative as packaging from renewable resources of the future. Utilization of byproduct egg albumen addresses the following issues:

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1. New uses for recovered egg albumen from the food industry

2. Improvement of technology for egg albumen processing

Preliminary results

1. Thermoplasticization of egg white proteins using compression yielded a sheet.

2. Decreasing the water content in the egg white network led to an improved visual appearance of the sheets.

3. Optimum processing temperatures for egg white proteins have been found to be between 130-150 °C.

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CHAPTER 2

LITERATURE REVIEW

2.1 Materials

2.1.1 BIOPOLYMERS

Biopolymers are polymers that are generated from renewable natural sources, are often biodegradable and nontoxic. They can be produced by biological systems such as microorganisms, plants and animals, or chemically synthesized from biological materials such as carbohydrates, starch and proteins (Flieger et. al. 2003). In order to convert raw materials into a useful resource for making biodegradable polymers, they have to be extracted from a plant or animal first. This process is then followed by a chemical or biotechnological process of monomer polymerization. Factors encouraging the adoption of biopolymer may be summarized to include (Song et al. 2011):

1) "Rapidly rising societal awareness of the environmental impact of food and packaging wastes, particularly, with regard to concerns over climate change and water availability"

2) "Public perception of packaging waste linked to the litter issue stresses the need for the ultimate disposal of used packaging materials in an ecologically sound manner to promote sustainability"

3) "Corporate social and environmental responsibility (CSER) commitment to initiatives actively supporting sustainable development and reducing carbon footprint"

4) "A means for retailers and manufacturers to further differentiate their brands and appeal to the consumer through the development of bioplastics that are both functional and offer superior carbon credentials"

5) "Trends in plastics packaging raw material and crude oil prices"

6) "Regulatory/environmental trends"

7) "Diversity/improvement in agro-economy through increased use of compost as a soil improved by providing benefits, such as increased carbon retention, improved water and nutrient retention, reduced need for additional chemical inputs, suppressing plant disease and increasing earthworm and micro-organism biomass as much as fivefold"

8) "Expanding supplier base worldwide and increasing rate of technical development"

Bioplastics are biodegradable plastics made from renewable resources (Stevens 2002). Although bioplastics may be a very desirable alternative to solve the environmental concerns of petroleum-based, non-biodegradable packaging plastics, they also have some drawbacks. One of the most significant drawbacks is the perceived competition with food production because it will influence the prices of food around the world. As a result, attention is shifting to "Second Generation" bioplastics which are manufactured from non-potential food sources (Verbeek & van den Berg, 2011). However, one of the challenges to commercialize these "Second Generation" bioplastics is to integrate them into common plastic processing methods, such as extrusion and compression. Some recent developments include the use for agro-industrial wastes for the production of bacterial PHA, and the use of natural low cost proteins (wheat gluten and soy) in plastic manufacture (Deng et al. 2006; Gallstedt et al. 2004; Lodha et al. 2005; Mangavel et al. 2004; Sun et al. 2007).

2.1.1.1 PROTEINS

Proteins are a renewable, biodegradable/edible resource with great potential to produce materials due to the diverse building blocks of proteins and their unique structures (Domenek et al. 2003). They are fundamental and integral food components, both nutritionally as a source of energy and amino acids and functionally in physiochemical and sensory aspects (Grappc et. al.

1998). Proteins consist of natural chains of α-amino acids joined by amide linkages and can be degraded by enzymes such as proteases (Clarimval & Halleux, 2005). They are classified as condensation polymers, because their synthesis involves elimination of water to produce a polypeptide (Verbeek & van den Berg, 2011). During heat processing, proteins disaggregate, denature, dissociate, unravel, and align in the direction of the flow. These changes allow the protein molecules to recombine and cross-link through specific linkages (Areas 1992; Redl et al. 1999). These cross-linking reactions can result in a high melt viscosity mixture which requires addition of plasticizers to increase free volume and mobility of molecules for easier processing. As temperature increases above the glass transition, the plasticized proteins turn into a soft, rubbery material that can be shaped into desired forms. Upon cooling, the matrix network gets fixed into the desired structure (Pommet el al. 2003). Proteins present good film-forming ability. Proteinous films generally exhibit meaningful mechanical properties, some of them comparable to those of petroleum-based films (Audic and Chaufer, 2005). Some protein-based films and coatings also present high barrier properties for gases such as O_2 and CO_2 (Chen 1995; Park 1999). Protein properties can be adjusted for the requirements of specific applications because their amino acid monomers that differ in side groups and can be modified by chemical, enzymatic and /or mechanical reactions to allow film formation or to improve film properties (Graapc & Kolster, 1998).

Proteins such as soy protein, wheat gluten, corn zein, and egg albumen, are renewable materials that are produced on an annual kiloton scale, and recent studies have shown their suitability for the manufacture of bioplastics (Gomez-Martinez et al., 2009; Kim, 2008; Mohanty et al., 2005; Tummala et al., 2006; Zheng et. al., 2003) Therefore, proteins have a huge potential to be a resource for manufacturing plastics.

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2.1.1.1.1 EGG WHITE

Protein	Amount of Albumen (% Dry Basis)	pI	Molecular weight (kDa)	Td ($^{\circ}$C)
Ovalbumin	54.0	4.5	44.5	84.0
Ovotransferrin	12.0	6.1	77.7	61.0
Ovomucoid	11.0	4.1	28.0	77.0
Ovomucin	3.5	4.5-5.0	$5.5\text{-}8.3 \times 10^3$	nd
Lysozyme	3.4	10.7	14.3	75.0
G2 globulin	4.0	5.5	49.0	92.5
G3 globulin	4.0	5.8	49.0	nd
Avidin	0.05	10.0	68.3	nd

pI - isoelectric point

Td = denaturation temperature

nd = not determined

Table 2.1. Major protein components of egg white and their fractions. (Adapted from Mine, 1995)

Egg yolk has a greater number of applications in the food industry as compared to egg white or albumen, leading to a surplus of egg albumen in the egg-breaking industry of North America (Gennadios et al., 1996). In Europe, there is also an overproduction of egg white due to the more than proportional egg yolk consumption (Van Dijk, T. 2008).

Egg white is a complex protein system consisting of ovomucin fibers in an aqueous solution of globular proteins (Powrie & Nakai, 1986). Ovalbumin constitutes more than half of egg white proteins (Table 2.1), and contains four free sulfhydryl (S-H) groups. Other major proteins that make up egg albumen are ovotransferrin, ovomucoid, and lysozyme. These proteins contain many disulfide (S-S) bonds. It has been hypothesized that the mechanism of film formation involves inter- and intra- molecular S-S bonds and S-H groups. Egg white can be easily produced in a dry powder form by desugaring (glucose removal) liquid egg white followed

by spray drying or some other drying technique (Bergquist., 1986). This dry form known as egg white protein powder can be used to produce films.

Most research has only been done on egg white-based films formed by "wet process" or solution-casting methods (Gennadios et.al., 1996; Handa et. al., 1999; Lim et al., 1998). Egg white-based films have been reported to be clearer and more transparent than wheat gluten, soy protein isolates and corn zein films (Froning 1998). Froning reported that egg white-based films could be used for water-soluble packets/pouches for ingredients in the food, chemical and pharmaceutical industries. Since extrusion is one of the most important polymer processing techniques in use today (Hernandaz-Izquierdo, 2008a), the extrusion of egg white proteins would be an important step over the "wet process" and toward enhancing the utilization these proteins in the processing industry. This would definitively increase egg white's commercial potential. The understanding of the interaction of plasticizers and moisture with proteins for an optimal formulation is a precursor step for the successful extrusion of the proteins to lead thermoplastics. This understanding has been obtained from compression molding studies and is available for proteins like whey protein, soy protein, wheat gluten and corn zein (Di Gioia and Guilbert, 1999; Ogale & Cunningham, 2000; Pommet and others 2005; Sothornvit et al. 2003). However, there is no study that covers the interaction of plasticizer and moisture content for optimal formulation of egg white proteins to lead thermoplastics made from such proteins.

2.1.2 PLASTICIZERS

Plasticizers are an important class of low molecular weight non-volatile compounds that are widely used in polymer industries as additives (Sejidov et al. 2005). The primary role of plasticizers is to improve the flexibility and processability of polymers by lowering a second

order transition temperature knows as glass transition temperature (Tg) (Vieira et al. 2011). The Tg of a polymer is a function of molecular motion and the motion of polymer chains can be described in terms of four types of motion (Kuma and Gupta 1998):

- "Translational motion of the entire molecule"

- "Long cooperative motion, allowing flexing and uncoiling"

- "Short cooperative motion"

- "Molecular vibration"

This is dependent on the chemical structure of the polymer in the following ways:

- *"Chain flexibility*. Bond rotation determines the overall flexibility of polymer chains; large bulky substituents hinder bond rotation, thereby increasing the Tg."

- *"Inter and intra-molecular forces*. Chain mobility is restricted by interactions between chain segments or between polymer chains."

- *"Molecular mass*. A lower molecular mass implies more chain ends per unit mass, resulting in more free volume. An increase in free volume implies a decrease in Tg."

- *"Cross-linking*. Cross-linking effectively restricts relative chain movement, thereby increasing the Tg."

Proteins typically have a very high Tg because most amino acids have large side chains that hinders bond rotation and decrease chain flexibility. It is possible to change or lower the Tg of proteins by the use of plasticizers. By lowering the Tg of a polymer, the temperature for the change of material to its rubbery-flow state is also reduced. Therefore, this allows the protein to be processed without excessive degradation with reasonable processing conditions (Verbeek & van den Berg, 2011). Plasticizers reduce the tension of deformation, hardness, density, viscosity

and electrostatic charge of a polymer, while at the same time, their presence increases polymer chain flexibility, resistance to fracture and dielectric constant (Rosen 1993).

There are two possible types of plasticizers: internal and external plasticizers. External plasticizers are substances with low volatility that are added to polymers externally. The molecules of the plasticizer then interact with polymer chains of the protein, but they are not chemically attached to them by primary bonds. Therefore, plasticizers can be lost by evaporation, migration or extraction after processing from the films or sheets. On the other hand, internal plasticizers are inherent parts of the polymer molecules and combines to become part of the product, which can be either co-polymerized into the polymer structure or reacted with the original polymer (Frados 1976). Internal plasticizers generally have bulky structures that provide polymers with more space to move around and prevent polymers from coming close together. Therefore, they soften polymers by lowering the Tg and, thus, reducing elastic modulus. A strong temperature dependence of material properties is observed for both plasticizers. The benefit of using external plasticizers, compared to internal plasticizers, is so that we can have the opportunity to select the right substance depending on the desired product properties (Sothornvit and Krochta 2005).

2.1.2.1 GLYCEROL

One of the most widely used plasticizers in thermoplastic processing of proteins is glycerol ($C_3H_8O_3$). This plasticizer is a low molecular weight, hydrophilic plasticizer (Ogale & Cunningham, 2000; Pommet et al. 2003; Pommet et al. 2005; Redl et al. 1999; Sothornvit et al. 2003; Sothornvit et al. 2005; Zhang et al. 2001). Glycerol's ability and ease to insert and position itself within the three-dimensional biopolymer network of the polymer results in a highly

plasticizing effect in protein polymers (Di Gioia and Guilbert 1999). From these studies, the critical factors for a good plasticizer were found to be: low melting point, low volatility, and protein compatibility. In addition, permanence in the film and amount of plasticizer needed should be taken into account when choosing a good plasticizer (Di Gioia and Guilbert 1999; Sothornvit and Krochta 2005).

2.1.2.2 WATER

Water is the most effective plasticizer in biopolymer materials, enabling them to undergo the glass transition, facilitating deformation and processasability of the biopolymer matrix. Without water addition, the temperature region of thermal degradation would be easily reached before films could be formed (Tolstoguzov 1993). The film's ability to attract water has been shown to affect the barrier properties of protein films (Sothornvit and Krochta 2005). Zhang and others reported that an increase in moisture content of their soy protein sheets resulted in significantly lower Tg, reflecting the plasticizing effect of water (Zhang et al. 2001).
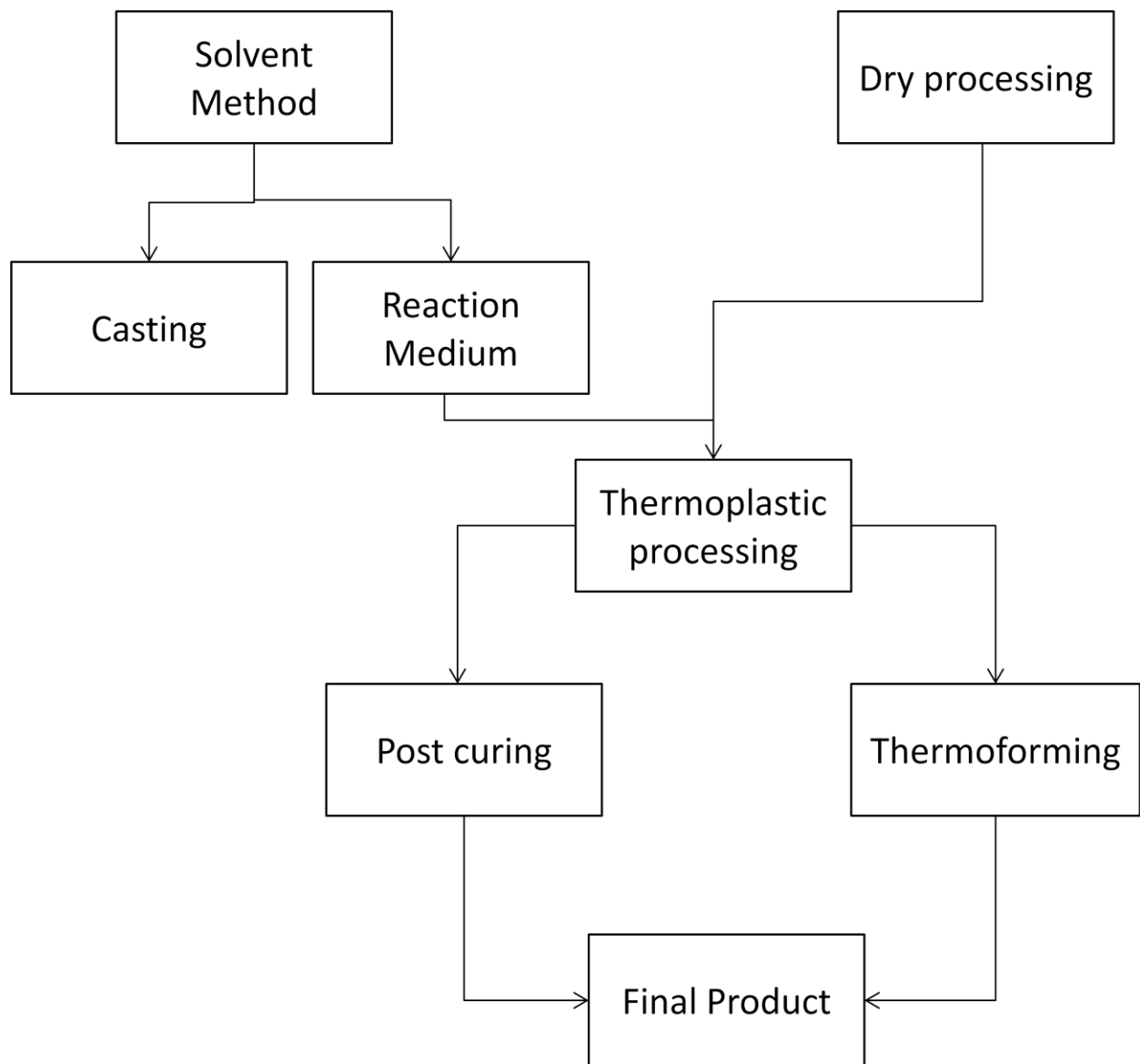
Figure 2.1. Processing paths leading to thermoplastic processing (Verbeek & van den Berg, 2009)

Formation of biodegradable films can be achieved by 2 main processes: the wet process and the dry process (Figure 2.1). The "wet process" involves biopolymer dispersion or solubilization in a film-forming solution followed by evaporation of the solvent. The "dry process" relies on the thermoplastic behavior that some proteins display at low moisture levels in compression and extrusion. (Cuq et al, 1997; Liu et al., 2006; Pommet et al., 2005) Therefore, the thermoplasticization of proteins based on the combination of heat, mechanical shear and suitable plasticizers during extrusion or compression is crucial to form products by traditional shaping methods like compression molding, thermoforming, calendaring, injection molding and film blowing (Ha and Padua, 2001; Wang et. al., 2005). Currently, studies have only been done to extrude wheat gluten (Redl et al., 1999), soy protein (Pommet et al., 2003; Zhang et al., 2001), whey protein (Hernandaz-Izquierdo, 2007) as films and corn zein as both foams (Salerno et. al., 2007) and films (Wang and Padua, 2003; Olivero et. al, 2010).

2.2.1 SOLUTION-CASTING

Most methods for preparing egg white protein films involve denaturation of egg white protein in aqueous solution, followed by a casting process whereby the film-forming solution is spread on a level surface and then dried to produce films (Lim et al. 2002). The first step of denaturation is achieved by adjusting solution to pH 10.5 to 11.5, and heating at 40°C for 30 minutes (Handa et al. 1999) The increase surface S-H concentration with thermal and alkaline denaturation allows formation of S-S bonding by oxidation and sulfhydryl-disulfide interchange reactions (Gennadios et al. 1996) and make the films more stretchable. Then the film formation can be obtained by solvent casting or by extrusion. Little has been published in this research area. However, there are several drawbacks associated with it (Verbeek & van den Berg, 2011):

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- "Large quantities of heat is required to evaporate the solvent"

- "Solvents may be expensive to use in large volumes"

- "Film thickness is limited"

- "Molded articles of complicated design cannot be made"

- "Specialized equipment needed for film casting"

Therefore it is important to explore common plastic processing methods, such as extrusion and injection molding, to integrate these bioplastics into them. Production of these bioplastics commercially on a large scale will save due to the economy of costs.

2.2.2 COMPRESSION MOLDING

Processing conditions dictate the type and extent of physical and chemical modifications that take place during thermoplastic processing of proteins (Moraru and Kokini 2003). Thermoplastic processing involves melting a polymer, followed by shaping and finally cooling the material in its new form. The softening temperature, atmospheric stability, as well as the geometry and size of the finished product are important (Callister 2003). Some important thermoplastic processing techniques are compression molding and extrusion. The combination of high temperatures, high pressures, short times, and low moisture contents in compression molding caused transformation of protein-plasticizer mixtures into viscoelastic melts. The protein films are then formed upon cooling through hydrogen, ionic, hydrophobic, and covalent interactions. (Pol et al. 2002) Protein-based films produced from compression molding have a wide range of mechanical and barrier properties that is dependent on factors such as formulation and processing conditions used. Thus, it is a suitable technology to investigate the thermoplastic properties of plasticized proteins as well as the properties of the resulting films. Compression

molding can also serve as a step toward the use of a more continuous, high-speed technology for film manufacture such as extrusion (Hernandez-Izquierdo et al., 2008b). Compression molding, extrusion and injection molding have been used to successfully produce proteinous bioplastics (Ralston 2005).

<p style="text-align:center">2.3 Mechanisms</p>

Proteins offer a large range of possible interactions and chemical reactions due to their various amino acids groups, and the properties of biopolymers derived from these proteins will depend largely on their primary structure. Some of the more important mechanisms during protein processing are denaturing, cross-linking and plasticization.

2.3.1 PLASTICIZATION

Typically, proteins and plasticizers are blended before they are thermally processed. As a result of blending, a highly viscous melt should be formed. Both the processing temperature and the viscosity of the protein network can be affected by the type and amount of plasticizer. Generally, increasing the amount of plasticizer will lower the processing temperature and viscosity of the blend (Verbeek & van den Berg, 2011).

The plasticizing effect of small polar molecules, such as glycerol and water, has been described in terms of insertion and positioning within the three-dimensional protein network earlier. The following plasticizing mechanisms have been proposed (Di Gioia and Guilbert 1999; Sothornvit and Krochta 2005):

a) "The lubricity theory, where the plasticizer is seen as acting as a lubricant to facilitate mobility of the chain molecules past one another."

<p style="text-align:center">22</p>

b) "The gel theory, which considers the disruption of polymer-polymer interactions (hydrogen bonds and van der Waals or ionic forces)"

c) "The free volume theory, which considers that the plasticizer increases the free volume and mobility of polymer chains. This theory has been used to understand the effect of plasticizers in lowering the glass transition temperature."

d) "The coiled spring theory, which explains plasticizing effects from the point view of tangled marcromolecules."

The mechanism of how plasticizers work is typically described using the free volume theory. Free volume is defined as the space between polymer molecules, and is dependent on temperature because of increased molecular motion. Then dilatometry is used to measure the temperature dependant change in specific volume, a sudden change in slope is observed over the glass transition region of the polymer. It is argued that the occupied volume increases uniformly with temperature, but, the discontinuity in total volume corresponds to the glass transition, indicating commencement processes that control visco-eleastic behavior (Ward and Hadley 1993). Free volume can be increased by increasing the number of chain ends, or rather, decreasing the molecular weight. External plasticizers increase the free volume by addition of a small molecule at any location along the polymer chain. They enable more chain movement, thereby reducing or decreasing Tg.

2.3.2 PROTEIN INTERACTION

Proteins can participate in chemical reactions through covalent (peptide and disulfide linkages) and non-covalent (ionic, hydrogen, and van der Waals) interactions. On top of that, hydrophobic interactions can also occur between the non-polar groups of amino acid chains

(Kokini et al. 1994). Upon addition of plasticizers, the proteins will aggregate and swell to become granules. It is thought that this aggregation of proteins involved sulfhydryl/disulfide interchange reactions. (Pommet et al. 2005)

Based on the structure of proteins and the requirements for thermoplastic processing, three broadly categorized processing requirements have been identified (De Graaf 2000):

- "Breaking in intermolecular bonds (non-covalent and covalent) that stabilize proteins in their native form by using chemical or physical means"

- "Arranging and orientating mobile chains in the desired shape"

- "Enabling formation of new intermolecular bonds and interactions to stabilize the three-dimensional structure"

These mechanisms are usually observed during the thermoplastic processing of proteins, and they combine to result in a cross-linked polymer that can impart the properties of a bioplastic. Different factors such as formulations, processing conditions (time, temperature and pressure) significantly affects each of these mechanisms at their respective stages, and produce protein sheets and films with varying thermal, mechanical and barrier characteristics. Therefore, it is important to study the interaction of protein during blending, processing and post-processing storage.

2.3.3 TEMPERATURE AND MOISTURE CONTENT EFFECT ON PROTEIN

Temperature and moisture also affect proteins during thermoplastic processing. High temperatures and low moisture contents can result in their degradation. Glass transitions of zein, gliadin, glutenin and whey proteins as a function of moisture content has been studied (Hernandez-Izquierdo et. al., 2008b; Kokini et al. 1994) and state diagrams were developed to

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predict protein behavior during processing. From a molecular point of view, the transformation from a glassy to a rubbery state corresponds to an increase in disorder, free volume, and mobility of the polymer molecules. These modifications in molecular organization result in variations of material physical properties such as thermal, mechanical, and dialectical properties (Cuq et. al., 1997). These modifications might be triggered by a combination of temperature and different moisture contents in the protein. Therefore, it is important to study the effects of temperature and moisture content and investigate their interaction with the protein molecules.

2.4 Mixture properties

2.4.1 THERMAL CHARACTERIZATION
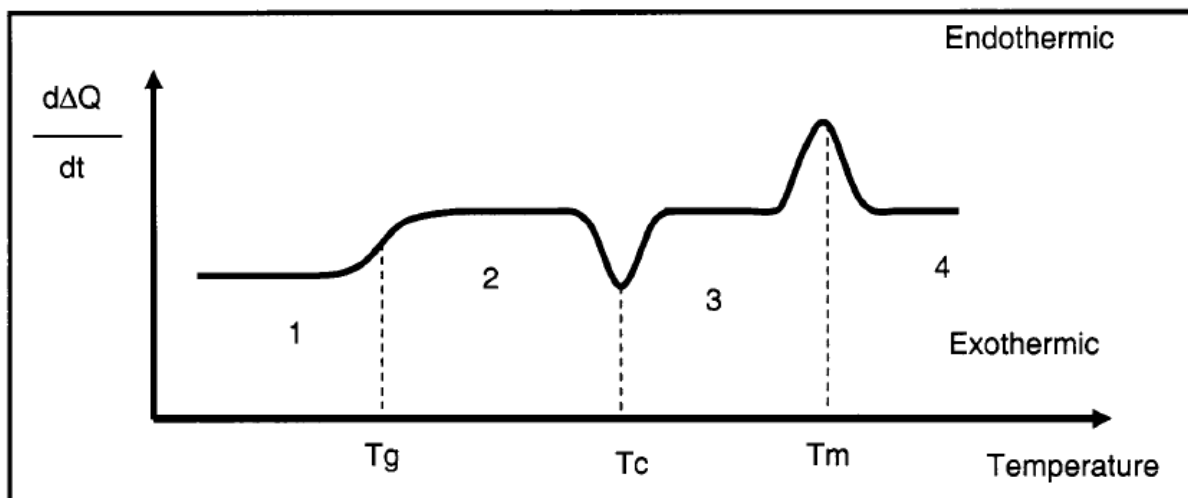
Figure 2.2. Idealized DSC thermogram (Adapted from Stevens, 2002)

Differential scanning calorimetry (DSC) is a widely used technique to characterize the thermal transitions of a polymer. Figure 2.2 shows an idealized DSC thermogram (Stevens 2002). The DSC thermogram shows the thermal transitions stages that an amorphous polymer in the glassy state would go through as it is heated. The first region shows the polymer in its glassy stage and as the temperature is increased, the polymer reaches its glass transition temperature

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(Tg). After passing through its Tg, the polymer changes to a rubbery state, where the molecules have gained some energy and weakened the interactions between the polymer chains. As more thermal energy is gained by the polymer chains, they may be able to re-arrange and crystallize at its temperature of crystallinity (Tc). If the continues to gain even more thermal energy, the crystalline domains in the polymer would melt at a characteristic melting temperature (Tm), changing from solid to liquid (Hernandez-Izquierdo et al. 2008a). The thermal properties of the protein mixtures can be affected by several factors, such as formulations which determine its moisture and plasticizer content.

Hernandez-Izquierdo and others (2008b) studied the effect of moisture content and glycerol of whey protein isolate-glycerol mixtures and found out that the glass transition temperatures were affected by both factors. They suggested that the hydrophilic nature of glycerol resulted in higher moisture contents, which in turn, contributed to a higher plasticization of the polymer matrix. They mentioned that the occurrence of the endothermic transition was essential for the transformation of the mixtures into a thermoplastic-extrudable melt. They used this information to determine the processing temperatures for their extrusion process.

Thermogravimetric analysis (TGA) thermograms provide information on the thermal stability (weight loss and rate of weight loss as a function of temperature), including thermal degradation of protein powders and mixtures. Studies have been done on soy protein isolate (SPI) and zein to investigate on the degradation temperatures and thermal stability profile of the powders and mixtures (Ogale & Cunningham, 2000; Oliviero et al., 2010).

2.5 Sheet properties

2.5.1 MECHANICAL PROPERTIES

It is well-known that the polymer architecture plays an important role on the mechanical properties, and consequently on the process utilized to modeling the final product (injection molding, sheet extrusion, blow molding, thermoforming, and film forming). The mechanical behavior of a polymer can be evaluated by its stress-strain characteristics under tensile deformation. The stress is measured in force per unit area while the strain is the dimensionless fractional length increase. (Selke et al. 2004)

Mechanical properties of compressed-molded soy protein (Ogale & Cunningham, 2000; Pol et al., 2002), whey protein (Sothornvit et al., 2003), and wheat gluten (Pommet et al., 2005) have been reported. It was found that as the glycerol content increased from 20% to 40% in compression-molded soy protein films, tensile strength of the films decreased from 15.8 to 1.6 Mpa and the percent of elongation of the films increased from 1.5% to 106%. Sothornvit and others (2003) found that with an increase in glycerol content from 40% to 50% in compression-molded whey protein films, the percent elongation increased from 85% to 94% and the tensile strength decreased from 8 to 4 MPa. It was also observed that the molding temperature and pressure did not affect film tensile properties significantly. Pommet and others (2005) studied the tensile properties of compression-molded wheat gluten-based sheets plasticized with water, glycerol, 1,4-butanediol, lactic acid and octanoic acid as a function of the glass transition temperature and found out that higher plasticizer concentrations resulted in lower Tg values and lower tensile strength at break.

2.5.2 THERMAL PROPERTIES

Thermal properties are the relationships between the polymer properties and temperature. Amorphous polymeric materials do not have melting points, buy they do have a glass transition temperature. Tg. This is defined as the freeing in (on cooling) or the unfreezing (on heating) of micro-Brownian motion of chain segments 2-50 carbon atoms in length in the amorphous regions of a material. This motion is often referred to as segmental mobility, and reflects the ability of a portion of a polymer molecule to change its position with respect to its neighbors (Selke 2004). The visco-elastic behavior of amorphous or semi-crystalline polymers can be divided into five different transitional stages, which are glassy, leathery, rubbery, rubbery-flow and viscous flow stage (Kumar and Gupta 1998). This transformation of the polymer from one stage to another is dependent on the temperature which the polymer structure is exposed. From heat, the molecules of the polymer gains energy to reduce the interactions between chains, therefore allowing relative movement of chains. Processing can only be done above temperatures when the polymer is at its rubbery-flow region (Verbeek & van den Berg, 2011). Most literature on proteinous bioplastics suggests that processing should be done above the protein's softening point, which most often corresponds to a temperature well above Tg. For semi-crystalline materials, the ratio of glass transition to melting temperature is often given by "Tg/Tm = 0.6" (McCrum et al. 1997).

Differential scanning calorimetry (DSC) is one of the most common thermal analysis techniques used in determining the glass transition temperatures of various protein-based films (Di Gioia and Guilbert 1999; Ogale & Cunningham, 2000; Zhang et al., 2001). Ogale and Cunningham reported the existence of multiple glass transition temperatures in compression-molded soy protein isolate-glycerol films using DSC technique. Zhang and others (2001) found

out that at the same glycerol content, the Tg of the protein sheet decreased with increasing moisture content from DSC analysis, showing the plasticizing effect of water. At low moisture contents, even with the addition of glycerol, the Tg of soy protein sheets was above room temperature. However, DSC techniques are not as sensitive as Dynamic Mechanical Analysis (DMA) in detecting transitional temperatures. Menard (1999) reported that the DMA technique is 10 to 100 times more sensitive to changes of the Tg than the DSC technique (Menard, 1999). Thus, DSC and DMA can be considered one of the more reliable techniques to determine the Tg values of protein sheets containing moisture.

2.5.3 MORPHOLOGY

Formulations and processing conditions used during the production of protein sheets and films are critical factors affecting their microstructural characteristics and morphology. Ogale and others (2000) used atomic force microscopy (AFM) and scanning electron microscopy (SEM) to study the surface texture of compression-molded soy protein films. They found out that surface characteristics of their films ranged from smooth and glassy for pure protein films, rough with scattered air bubbles for 20% glycerol films, homogenous for 30% glycerol films to non-homogenous for 40% glycerol films (Ogale & Cunningham, 2000). The cross-sections of the films containing 0 and 20% glycerol were smooth, while 30 and 40% glycerol films presented ridges and valets, which they related the soy proteins films to being a more ductile material. Thus, investigation on the morphology of the protein mixtures and sheets can lead us to better understand the interaction between the proteins and the plasticizers.

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CHAPTER 3

**UNDERSTANDING THE EFFECTS OF GLYCEROL AND WATER ACTIVITY ON
THE THERMOPLASTICIZATION OF EGG WHITE PROTEINS**

Abstract

Protein-based bioplastics are a new generation of plastics. They result from the thermoplasticization of proteins, which requires heat and plasticizers. In this study, the effects of plasticizer type (water and glycerol (GLY)) and amount (0.34, 0.48 and 0.64 water activity and 0, 35, 40 and 45% GLY) on the thermoplasticization of the egg white protein (EWP) have been investigated using thermal studies (differential scanning calorimetry and thermogravimetric analysis), gravimetric analysis (moisture content determination), and morphological studies (scanning electron microscopy). The results show that the combination of 35% GLY content and 0.48 water activity yields an EWP network with a moisture content of about 12% and that this is sufficient to lower its second second-order transition temperature below 170°C, the temperature that marks the onset of thermal degradation of the EWP network. Under these conditions the EWP network can be thermoplasticized at 150°C with less than 15% degradation. The degradation can be reduced further by increasing the GLY content. This study provides data useful for the appropriate compounding as a precursor step for the manufacture of EWP-based bioplastics using industrial processes like compression and extrusion.

Industrial Relevance: Protein-based bioplastics are suitable to be produced commercially on a large scale using the same manufacturing methods as petroleum-based plastics (e.g., compression and extrusion). However, proteins require thermoplasticization prior to undergoing these thermal processes. Protein thermoplasticization is affected by the degree of compatibility between protein and plasticizer as well as the amount of plasticizer tolerated by the protein network. The thermoplasticization of EWP is affected by the type and amount of plasticizer (glycerol and water) which influence water absorption capability, first and second second-order transition temperatures, protein degradation temperature, and protein-plasticizer mixture homogeneity. The

appropriate combination of glycerol and water necessary for an optimal thermoplasticization of EWP has been identified in this study. These findings provide data useful for the manufacture of EWP-based bioplastics using industrial processes like compression and extrusion.

Keywords: egg white protein, thermoplasticization, glycerol, water activity.

3.1. Introduction

Protein-based bioplastics are a new generation of plastics. Bioplastics have advantages over petroleum-based plastics, like being made from renewable feedstock as well as biodegradability and compostability (Flieger, Kantorova, Prell, Rezanka, & Votruba, 2003; Siracusa, Rocculi, Romani, & Rosa, 2008). Protein-based bioplastics are suitable to be produced commercially on a large scale using the same manufacturing methods as petroleum-based plastics (e.g., compression and extrusion) (Cunningham, Ogale, & Acton, 2000; Gonzalez-Gutierrez, Partal, Garcia-Morales, & Gallegos, 2011; Hernandez-Izquierdo, Reid, McHugh, Berrios, & Krochta, 2008a; Sothornvit, Olsen, McHugh & Krochta, 2007; Zhang, Mungara, & Jane, 2001). However, proteins typically decompose at temperatures below their respective melting points due to their strong intra- and inter-molecular interactions including hydrogen bonds, van der Waals forces, and ionic bonds (Verbeek & van dan Berg, 2011). Therefore, they require thermoplasticization prior to undergoing the aforementioned thermal processes. Protein thermoplasticization requires the combination of heat and suitable plasticizers in order to lessen interactions. Heat causes the denaturation of the proteins, that is, their unfolding resulting from the loss of quaternary, tertiary and secondary structures, and consequently, reduces the interactions between the protein chains (Arntfield, Ismond, & Murray, 1990). Plasticizers act as internal lubricants. They decrease the interactions and attractions in the three-dimensional protein network leading to an increased free volume and molecular mobility (Sears & Darby, 1982). This allows the formation of a melt flow at temperatures that do not cause protein degradation and enables the processing of the proteins. From a molecular point of view, the transformation from a glassy to rubbery state results from an increase in disorder, free volume, and mobility of the polymer molecules (Hernandez-Izquierdo & Krochta, 2008b). Protein

thermoplasticization is affected by the degree of compatibility between protein and plasticizer as well as the amount of plasticizer tolerated by the protein matrix (Orliac, Rouilly, Silvestre, & Rigal, 2003). Glycerol is a low molecular weight, hydrophilic plasticizer which has been widely used in the thermoplasticization of proteins (Pommet, Redl, Guilbert, & Morela, 2005; Sothornvit et al., 2007; Zhang et al., 2001). Its high plasticizing effect has been attributed to its small size that allows it to interpose easily within the three-dimensional protein network (di Gioia & Guilbert, 1999). Water is also commonly used as a plasticizer for proteins (di Gioia & Guilbert, 1999; Slade & Levine 1993; Sobral & Habitante, 2002). Its effect has been attributed to a decrease in molecular interaction density for the protein network due to the replacement of protein-protein bonds with protein-water bonds (Cuq, Gontard, & Guilbert, 1997). Both, glycerol and water, have been reported to interact with readily accessible polar amino acids due to their polarity (di Gioia & Guilbert, 1999).

The efficacy of the plasticizer amount and type on the protein thermoplasticization can be determined by studying the second-order transitions that the proteins suffer when exposed to increasing temperature. Second-order transition temperatures are commonly determined using differential scanning calorimetry (Rouilly, Orliac, Silvestre, & Rigal, 2001). They depend on the protein structure and can easily be modified by the presence of plasticizers. The changes in second-order transition temperatures caused by plasticizers provide a better understanding of the interaction between protein matrix and plasticizer and therefore, can be used to predict protein thermoplasticization during processing. Thus, previous knowledge of these changes is required for an adequate manufacture of protein-based bioplastics using thermal processes like compression and extrusion. Currently, there are only a few studies focused on understanding the effect of the plasticizer content and type on the thermoplasticization of proteins. Most of them

are focused on either plasticizer content or plasticizer type but not on both of them (Cunningham et al., 2000; di Gioia and Guilbert, 1999; Hernandez-Izquierdo et al., 2008a; Orliac et al., 2003; Rouilly et al., 2001).

One source of protein for which the potential for the production of films and sheets has been demonstrated is the egg white protein (Gonzalez-Gutierrez, Partal, Garcia-Morales, & Gallegos, 2010; Gonzalez-Gutierrez et al., 2011; Jerez, Partal, Martinez, Gallegos, & Guerrero, 2007). However, these studies are only limited to one glycerol and moisture content. The aim of the present study is to understand the effects of glycerol and moisture contents on the thermoplasticization of egg white proteins in order to provide data useful for the successful compression and extrusion of egg white proteins and other protein-based bioplastics.

3.2. Materials and methods

3.2.1 Materials

Desugared, spray dried egg white protein (EWP) powder (minimum 92% solids, minimum 80% protein) was obtained from Rose Acre Inc. (Seymour, IN, United States). Glycerol (GLY) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, Unites States). Magnesium chloride ($MgCl_2$) and sodium nitrate ($NaNO_3$) were supplied by Columbus Chemical Industries Inc. (Columbus, WI, United States). Standard saturated salt solutions of 0.30 ± 0.01 and 0.50 ± 0.01 water activity were purchased from Decagon (Decagon Devices Inc, Pullman, WA, USA).

3.2.2 Methods

3.2.2.1 Proximate analysis

Proximate analysis was conducted according to AOAC Intl. (2000) procedures (method 992.15) to verify the composition of EWP powder. The resulting values were compared with the specifications provided by Rose Acre Inc. (Seymour, IN, United States).

3.2.2.2 Formulation and preparation of EWP-GLY-water mixtures

EWP-GLY-water mixtures with GLY contents of 0, 35, 40 and 45 % on dry basis (w/w) were prepared by intensive mixing of EWP and GLY for 10 minutes using a mortar and pestle, and posterior conditioning to water activities of 0.34 ± 0.01, 0.48 ± 0.02, and 0.64 ± 0.02 at 23.1 ± 0.1 °C. The conditioning was performed by placing the mixtures on aluminum dishes and then storing these for three days in closed environments maintained at different relative humidities (RH). Closed storage containers containing saturated solutions of $MgCl_2$ and $NaNO_3$ were used to condition the mixtures to 34 % and 64 % RH (0.34 ± 0.01 and 0.64 ± 0.01 water activity, respectively). A room conditioned at $55 \pm 5\%$ RH was used to condition the mixtures to 0.48 ± 0.02 water activity. Three replicates were prepared per GLY content and per conditioning condition. The water activity of the mixtures was verified using an AquaLab model CX-1 (Decagon Devices Inc, Pullman, WA, USA). The instrument was allowed to warm up for one hour and then calibrated with standard saturated salt solutions of 0.30 ± 0.01 and 0.50 ± 0.01 water activity.

3.2.2.3 Moisture content determination

The moisture content of the EWP powder and of EWP-GLY-water mixtures resulting from combinations of 0, 35, 40 and 45 % GLY and 0.34, 0.48 and 0.64 water activity was measured according the AOAC Intl. (2000) (method 927.05). Weight measurements were taken after 24 h by removing the samples from a vacuum oven (100 ^{o}C and 100 mmHg vacuum), and equilibrating these to room temperature inside a desiccator.

3.2.2.4 Thermal characterization

3.2.2.4.1 Differential Scanning Calorimetry

The second-order transition temperatures of the EWP-GLY-water mixtures described above were determined by using a differential scanning calorimeter (DSC Q100; TA Instruments, Newcastle, DE) with a liquid nitrogen cooling system. An amount between 7 to 10 mg of each type of mixture was hermetically sealed in an aluminum pan (TA Instruments, Newcastle, DE, USA), equilibrated to 0 °C, and then heated to 200 °C at a rate of 20 °C/min. During each run, the DSC cell was flushed with nitrogen at 70 ml/min to maintain an inert environment. The instrument was calibrated using pure indium. TA analysis software was used for data analysis in accordance with ASTM D3418 (ASTM 2010).

3.2.2.4.2 Thermogravimetric analysis

The decomposition temperature of the EWP-GLY-water mixtures resulting from combinations of 0, 35, 40 and 45 % GLY and 0.34 and 0.48 water activity was determined by using a thermogravimetric analyzer (TGA Q50; TA Instruments, Newcastle, DE, USA). An amount between 7 to 10 mg of each EWP-GLY-water mixture was placed in an aluminum pan (TA Instruments, Newcastle, DE, USA) and then heated from 25 to 300 °C at a rate of 20

°C/min. The percentage of weight loss of each sample as a function of temperature under a nitrogen-air (40 % - 60 %) atmosphere was analyzed.

3.2.2.5 Morphological characterization

2.2.5.1. Macromorphological characterization

The macromorphology of the EWP-GLY-water mixtures (35, 40 and 45 % GLY and 0.48 water activity) was observed using a Digital Camera PowerShot SD 600 (Canon). Photographs show an area of 2 cm x 1.5 cm of the surface of each of the mixtures.

3.2.2.5.2. Micromorphological characterization

The micromorphology of the EWP-GLY-water mixtures described above was observed using a Carl Zeiss Variable Pressure Scanning Electron Microscope (VP-SEM) EVO LS25 (Carl Zeiss Inc., Thornwood, NY, USA) equipped with a LaB6 gun. Nitrogen at a pressure of 30 Pa was used. Micrographs of cross sections of uncoated mixtures were collected using a beam energy of 10 kV. Micrographs were taken at 200x magnification and show an area of 1200 μm x 1600 μm (height x width). VP-SEM was used instead of high pressure SEM to prevent GLY from leaking due to the high pressure and to minimize damage caused by charging and coating. Thus, the mixtures were kept as close to original as possible.

3.2.2.6 Statistical analysis

A two-factor completely-randomized experimental design was used to study the effect of glycerol content (0, 35, 40 and 45 %) and water activity (0.34 ± 0.01, 0.48 ± 0.02 and 0.64 ± 0.02) on the thermal transitions and moisture contents of the EWP-GLY-water mixtures. The

statistical software Minitab 15 (Minitab Inc, State College, PA, USA) was used to perform a one-way analysis of variance (ANOVA; Tukey test; $p \leq 0.05$). Three different EWP-GLY-water mixtures per type of mixture were tested.

3.3. Results and discussion

3.3.1 EWP composition

The EWP powder composition was determined as 82.1 ± 0.61 % protein, 0.37 ± 0.17 % fat, 6.04 ± 0.20 % ash, and 3.51 ± 0.22 % moisture by proximate analysis and was consistent with the specifications provided by Rose Acre Inc. (Seymour, IN, United States).

3.3.2 Moisture content of EWP-GLY-water mixtures

Proteins can be hydrophobic or hydrophilic depending on the polarity of their side chains. Ovalbumin, the main protein found in egg whites, is considered to be hydrophilic. Table 3.1 list the moisture contents (%) of EWP-GLY-water mixtures with GLY contents of 0, 35, 40 and 45 % and pre-conditioned to water activities of 0.34 ± 0.01, 0.48 ± 0.02, and 0.64 ± 0.02 at 23.1 ± 0.1 °C. In general, the moisture contents of EWP-GLY-water mixtures were vastly different and ranged between 3 and 16 %. The large differences between moisture contents was caused by the different GLY contents and water activities present in the mixtures as both, GLY and water activity, had an effect on moisture content as discussed below. As the GLY content increased from 0 to 35 %, the moisture content of the EWP-GLY-water mixtures increased significantly ($P \leq 0.05$) (from 2.89 to 5.83 %, 8.39 to 10.9 % and 9.25 to 15.5 % for mixtures pre-conditioned to water activities of 0.34 ± 0.01, 0.48 ± 0.02, and 0.64 ± 0.02, respectively). This shows how the presence of GLY increases the water binding capabilities of the EWP-GLY mixtures. This might

be due to the hydrophilic nature of GLY, which increases the ability of the EWP-GLY mixture to absorb water from the environment. These results are in agreement with those reported for whey protein isolate (WPI)-GLY mixtures pre-conditioned to 0.34 water activity where an increase in GLY from 0 to 30 % increased the moisture content of the samples from 7.30 to 12.08 % (Hernandez-Izquierdo et al., 2008a). However, the moisture contents of EWP-GLY mixtures were lower than those reported for WPI-GLY mixtures at the same water activity which may be indicative of a lower affinity for moisture of EWP-GLY mixtures. This could be explained by a different positioning of the GLY molecules between the EWP and WPI chains caused by the different protein composition and therefore, the different three-dimensional structures. Beta-lactoglobulin is the main protein present in WPI (~ 65%) while ovoalbumin is the main one present in EWP (~ 54%). As the GLY content increased in the EWP-GLY mixtures from 35 to 40 %, their moisture content remained about the same ($p > 0.05$) (5.83 vs 5.79 %, and 15.5 vs. 16.2 % for 0.34 and 0.64 water activity, respectively), or slightly increased ($p \leq 0.05$) (10.9 vs 12.4 %) for a water activity of 0.48. This could be attributed to the interaction of water and glycerol with the protein matrix reaching a maximum. This seems to be supported by the fact that EWP-GLY mixtures with GLY contents higher than 40 % showed a decrease in moisture content. This decrease in moisture content could result from GLY becoming less effective in absorbing water beyond a certain threshold in the mixtures due to interactions between the GLY molecules which would reduce the number of OH groups binding water. Since non-significant differences were observed for some of the 40 and 45 % GLY mixtures, GLY contents higher than 45 % would need to be tested to determine if GLY contents higher than 40% produce a decrease in moisture content in EWP-GLY mixtures. These results differ from those reported for WPI-GLY mixtures pre-conditioned to 0.34 water activities where an increase in GLY from 30

to 50 % increased the moisture content of the WPI-GLY mixtures (Hernandez-Izquierdo et al., 2008a).

The capability of the EWP-GLY mixtures to absorb water from the environment was also affected by the water activity to which the mixtures were pre-conditioned. As observed in Table 3.1, the mixtures pre-conditioned to a water activity of 0.64 were the ones capable of absorbing more water from the environment. This could be explained by an increase in the free volume of the matrix caused by the interaction of the water with the EWP. Water has been reported to decrease the molecular interaction density of the protein network due to the replacement of protein-protein bonds to protein-water bonds (Cuq et al., 1997). The higher the water activity, the larger the free volume and as a consequence, the greater the water absorption from the environment. From these results, it can be concluded that both, the amount of glycerol and the water activity used during conditioning, play a key role in the capability of mixtures based on EWP to absorb water, which, in turn, contributes to the plasticization of the EWP as shown below.

3.3.3 Second-order transitions of EWP-GLY-water mixtures

Proteins typically have a very high second-order transition temperature (glass transition temperature (Tg)) because most of their amino acids have large side chains, hindering bond rotation and thereby decreasing chain flexibility. In addition, several of these amino acids lead to a number of possible intermolecular forces, thus further increasing Tg (Verbeek & van den Berg, 2011). Consequently, plasticizers are needed to lower Tg and to allow the processing of proteins without excessive degradation using reasonable processing conditions (di Gioia & Guilbert, 1999). Second-order transition temperatures can be measured using differential scanning

calorimetry (DSC). These transition temperatures can be identified as endothermic peaks in the DSC thermograms and result from an energy take-up. The DSC thermograms (first cycle) of some of the EWP-GLY-water mixtures (GLY content (0 and 45 %) and conditioning water activities (0.34 ± 0.01, 0.48 ± 0.02 and 0.64 ± 0.02) at 23.1 ± 0.1 °C) are presented in Figure 3.1 These thermograms show that EWP-GLY-water mixtures can have two different second-order transition temperatures. The first second-order transition temperature (first peak) can be attributed to the change of the protein matrix from rigid and brittle to rubbery that is caused by the alteration of the three-dimensional structure of the proteins forming the EWP due to the breakage of their stabilizing inter- and intra-molecular bonds (from folded proteins to unfolded proteins). This change was possible due to the presence of enough vibrational (thermal) energy in the system to create the sufficient free volume to permit sequences of the EWP to move. Denaturation of EWP caused by heat has previously been reported in the literature. Van der Plancken, Loey, & Hendrickx (2005) found three different peaks in thermograms obtained by DSC of EWP. These peaks were attributed to the desaturalization of ovotransferrin, lysozyme, and ovoalbumin. The DSC thermograms of the EWP-GLY mixtures show only one peak in the same temperature interval and this matches with the one attributed to the denaturation of the ovoalbumin (Van der Plancken et al., 2005). Peaks for ovotransferrin and lysozyme were not observed possibly because these proteins were denatured by the shear occurring during the mixing process. The second peak or second second-order transition observed at more elevated temperature could be attributed to the change of the protein matrix from rubbery to a viscous rubbery flowing state as a consequence of the creation of more free volume with the increase of the temperature and the presence of plasticizers (GLY and water molecules) which permitted more movement of the protein chains. The formation of this viscous rubbery flowing state is

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indicative of protein thermoplasticization and allows its processing. The unfolding of the proteins and the posterior chain movement caused by heat and plasticizers allows the recombination of the protein chains and their cross-linking, which, in turn, produces high molecular weight polymer chains. The cross-linking happens through specific linkages caused by groups like the sulfhydryl groups which are now being exposed due to the denaturation of the proteins. The first second-order transition temperature of the thermoplasticized EWP (86 to 89°C) is also higher than the glass transition temperature of the commercially available bioplastic polylactic acid which is around 60 °C (Joo, Auras, & Almenar, 2011). This higher first second-order transition temperature gives thermoplasticized EWP a performance advantage over materials made from other bioplastics.

3.3.3.1 First second-order transition temperature of EWP-GLY-water mixtures and denaturation of EWP

The first second-order transition temperatures of all of the EWP-GLY-water mixtures under investigation are presented in Figure 3.1 and Table 3.2. These first second-order transition temperatures ranged between 86 and 116 °C and can be attributed to the denaturation of the ovalbumin as discussed above. Similarly, Van der Plancken et al. (2005) found a peak at around 84 °C in the DSC thermograms of EWP.

The first second-order transition temperature of the EWP-GLY-water mixtures was affected ($p \leq 0.05$) by the water activity to which the mixtures EWP-GLY were pre-conditioned. The higher the water activity the lower the first second-order transition temperature (from 116 to 113 °C for a moisture content change from 2.89 to 9.25 %). This agrees with the observed reduction of the first second-order transition temperature (from 180.8 to 5.3 °C) of sunflower

protein isolate (SFPI) caused by the increase of water (0 to 26.12 %) resulting from the water absorbing capability of the SFPI (Rouilly et al., 2001). Comparable results have been obtained with other types of proteins (Morales & Kokini, 1997). However, water activity had a much lower impact on the first second-order transition temperature of the EWP-GLY-water mixtures than GLY content as observed by the much lower first second-order transition temperature obtained when GLY was present. This may be due to the significant increase in the moisture content of the mixtures caused by the GLY. Thus, the increase in moisture contents from 2.89 to 5.83 %, from 8.39 to 10.9 % and from 9.25 to 15.5 % caused by the presence of GLY in the mixture pre-conditioned to water activities of 0.34 ± 0.01, 0.48 ± 0.02 and 0.64 ± 0.02, respectively, resulted in a reduction ($p \leq 0.05$) of the first second-order transition temperature from 116.2 to 89.3 °C, 114.1 to 88.3 °C and 113.0 to 87.5 °C, respectively. This significant reduction in the first second-order transition temperature was also observed in WPI-GLY-water mixtures with similar GLY contents and water activity conditioning conditions (Hernandez-Izquierdo et al., 2008a). However, the reduction was lower for EWP-GLY-water mixtures. This could be explained by the lower moisture content of the EWP-GLY-water mixtures compared to that of the WPI-GLY-water mixtures. This suggests that although the presence of GLY as a plasticizer has an immense role in the plasticization of proteins its effectiveness depends on the type of protein. A GLY content of 35% or higher in the EWP-GLY-water mixtures resulted in very little decrease or no decrease of the first second-order transition temperature for water activities of 0.48 and 0.64 (Table 3.2). These results are in agreement with those of moisture content (Table 3.1) as the moisture content remained about the same for GLY contents higher than 35 %.

3.3.3.2 Second second-order transition temperature of EWP-GLY-water mixtures and thermoplasticization of EWP

Figure 3.1 and Table 3.2 show that GLY and conditioning water activity have an effect on the thermoplastifcation of EWP. GLY is needed for the thermoplasticization of the polymer matrix as a second second-order transition, which indicates the formation of a viscous rubbery flowing state, only occurs when GLY is present in the mixture (Figure 3.1). Without GLY, the protein matrix degrades having only a first second-order transition as a second one is not observed before 200 °C (Figure 3.1), at which temperature a marked degradation starts for EWP as shown in the TGA results (Figure 3.2). In this regard, direct and indirect effects can be attributed to GLY. The direct effect results from its distribution among the protein chains while the indirect one results from the water molecules gained by its presence. Both GLY and water molecules increase chain separation and mobility, which in turn facilitate the formation of the viscous rubbery flowing state, that is, the thermoplasticization of the EWP. Once the GLY is present in the EWP-GLY mixture, its increase seems not to have an effect on the second second-order transition since no significant ($p > 0.05$) differences between second second-order transition temperatures were observed for mixtures with GLY contents ranging between 35 to 45 % (Table 3.2). These results are in agreement with those of moisture content (Table 3.1) as the moisture content remained about the same for GLY contents higher than 35 %. The water activity to which the mixtures were pre-conditioned also played an important role in the thermoplasticization of the EWP. As shown in Figure 3.1, the onset of the second second-order transition temperature decreased and the peak became deeper and broader with increasing water activity. These lower second second-order transition temperatures obtained with the increase in conditioning water activity from 0.34 to 0.48 or greater are listed in Table 3.2. This decrease in

transition temperature may result from the higher moisture content in the polymer matrix since the mixtures pre-conditioned to high water activities (0.48 and 0.64) were the ones capable of absorbing more water from the environment (Table 3.1). The effect of the water activity became more evident at the lowest GLY content. This shows the importance of the presence of water molecules for the thermoplasticization of the EWP since this lower temperature is necessary for the second second-order transition to happen and to enable EWP thermoplasticization without excessive protein degradation, as supported by the TGA results. For example, Figure 3.1 and Table 3.2 show a second second-order transition temperature at about 170 °C for EWP-GLY-water mixture containing 45 % GLY and pre-conditioned to 0.34 which is about the same temperature at which the mixture starts to degrade markedly (Figure 3.2b). The increase in water activity from 0.34 to 0.48 lowered this second second-order transition temperature by about 30 °C and therefore, the second-order transition temperature needed to obtain a viscous rubbery flowing state was far from the temperature at which the mixture starts markedly to degrade. In agreement, Tolstoguzov (1993) reported that the temperature region of thermal degradation of proteinous mixtures without water would be easily reached before films could be formed from the mixtures. Water is known to be one of the most effective plasticizers for biopolymers. It enables them to reach a lowered Tg and facilitates their deformation and processability (Hernandez-Izquierdo & Krochta, 2008b). Thus, a successful thermoplasticization of EWP can be achieved by using an adequate amount of plasticizer, moisture content and temperature. In agreement, Hernandez-Izquierdo et al. (2008a) reported that for WPI-GLY mixtures containing high GLY content and high conditioning water activity (50 % and 0.48, respectively), the additional endothermic transition detected was essential for their transformation into a thermoplastic-extrudable melt. The onset of the first second-order transition temperature also

decreased and its peak became deeper and broader with the addition of GLY and the increase in water activity (Figure 3.1). However, the effect of these on the first second-order transition temperature was less pronounced than that on the second one and therefore, GLY and water activity had less effect of interacting when the temperature is lower and consequently, there is less free volume for GLY and water molecules to interact with the polymer matrix.

3.3.4 Degradation of EWP-GLY-water mixtures

Figure 3.2a and b (TGA thermograms) provide information on the thermal stability (weight loss and rate of weight loss as a function of temperature), including thermal degradation of EWP powder and of EWP-GLY-water mixtures. Figure 3.2a shows that the EWP-GLY mixtures with GLY contents of 35 and 40 % and water activity of 0.48 exhibited a weight loss of less than 15 % at temperatures around those necessary for the EWP thermoplasticization and therefore, it is possible to process the mixtures without excessive degradation. A subsidence was observed for the protein mixture without GLY at temperatures below 100 °C but this could be attributed to water loss instead of to protein degradation. A similar subsidence was observed for other proteins like soy protein isolate (SPI) and zein at 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 weight loss of the protein mixture without plasticizer was relatively small between 100 to 200 °C, moderate between 200 and 260 °C and significant above 260 °C (Figure 3.2a). In contrast, the mixtures containing GLY showed a moderate weight loss even at very low temperatures which rose significantly above temperatures around 170 °C (Figure 3.2b). Therefore, 170 °C could be established as the upper limit for thermal processing of EWP-GLY mixtures. Among the EWP-GLY mixtures, an effect of the GLY content on their thermal

degradation was observed. The mixture with lower GLY content (35 %) lost about 5% more weight than the one with higher GLY content (45 %) (Figure 3.2a). The rate of decomposition for EWP-GLY mixtures with 35% GLY was the fastest, followed by that of the mixtures with 40% GLY and finally, by that of the mixtures with 45% GLY as shown by the increase in height of the peak in the derivative thermogravimetric curve (Figure 3.2b). This greater weight loss could be explained by the instability of the water which is not bounded to the GLY. Therefore, a higher GLY content resulted in a more stable polymer matrix, showing a smaller percentage of weight loss. Non-bounded water has been attributed to be most probably the reason for a faster decomposition rate of other bio-based polymers like polylactic acid when blended with cyclodextrins (modified starch) which contain some moisture even after these have been dried (Joo et al., 2011).

3.5 Macro- and micro-morphology of EWP-GLY mixtures

Figure 3.3 shows the photos of EWP-GLY-water mixtures differing in GLY content (35, 40 and 45 %). The EWP-GLY-water mixtures containing 35 % GLY had fine granular consistency (Figure 3.3a) while the ones with 40 % GLY had larger particle size (Figure 3.3b), and those with 45 % GLY (Figure 3.3c) were a homogenous viscous mass. These consistencies were observed for EWP-GLY mixtures pre-conditioned to either 0.34 ± 0.01 (photos not shown) or 0.48 ± 0.02 water activities (photos shown). These consistencies agree with the ones reported by Sothornvit et al. (2007) for WPI-GLY mixtures with 30, 40 and 50 % GLY. The EWP-GLY mixtures pre-conditioned to 0.64 ± 0.02 water activity presented an excess of water (photos not shown) and these make them too sticky to be processed. Therefore, based on these results and the

moisture content results, about 12 % seems to be the maximum moisture content necessary for the adequate processing of EWP-GLY mixtures at the GLY contents tested in this study.

Figure 3.4 shows the VP-SEM micrographs of a cross section of each of the EWP-GLY-water mixtures shown in Figure 3.3. The mixture with 35 % GLY contained more and larger air pockets (Figure 3.4a) than the mixtures with higher GLY contents (Figures 3.4b and 3.4c). In addition, the mixture with the lowest GLY content had a rough texture (Figure 3.4a) while the mixture with the highest GLY content had a smooth texture (Figure 3.4c). The mixture containing 40 % GLY had a combination of both textures but with a higher proportion of smooth texture (Figure 3.4b). These results suggest that the increase of glycerol in the EWP-GLY-water mixtures results in a better mixing between protein and plasticizer, which in turn, yields smaller air pockets and in a more smooth texture of the mixture. This could be explained by the GLY swelling the EWP network. GLY can interpose easily between the EWP network and disrupt the protein-protein interactions. The higher the GLY content the more protein-protein interactions disrupted and the more the partial unfolding (loss of quaternary structure) and consequently, the higher the swelling capacity of the EWP network. The observed better mix seems not to have an effect on the second-order transition temperature of the mixtures but affects their rate of decomposition.

3.4. Conclusions

The results of this study show that the combination of GLY and water at specific levels can optimize the thermoplasticization of EWP. The compounding of EWP with GLY and water increased the capability of the EWP network to absorb water in sufficient amounts to allow the lowering of the second second-order transition temperature of the EWP network below the

marked thermal degradation of EWP network. GLY was necessary for the thermoplasticization to occur but its increase from 35 to 45 % did not have a significant effect on the capability of the EWP network to absorb water and consequently, on the second second-order transition temperature. However, the increase of GLY in the EWP network improved the mixing between protein and plasticizer and reduced the EWP rate of decomposition. The increase in water activity (from 0.34 to 0.64) increased the capability of the EWP network to absorb water and consequently, lowered the second second-order transition temperature. A EWP network with a GLY content of 35 % and a water activity of 0.48 can be thermoplasticized at 150 °C without excessive degradation (less than 15 %). A maximum temperature of 170 °C and a maximum moisture content of about 12 % can be established for the adequate thermoplasticization of EWP. This study provides data useful for the appropriate compounding as a precursor step for the processing of EWP using industrial processes like compression and extrusion in order to develop films and sheets made from egg whites.

Acknowledgements

APPENDIX

GLY content (%)	Moisture Content (%)		
	a_w of 0.34	a_w of 0.48	a_w of 0.64
0	$2.89 \pm 0.06^{Bc*}$	8.39 ± 0.02^{Cb}	9.25 ± 0.12^{Ca}
35	5.83 ± 0.68^{Ac}	10.9 ± 0.33^{Bb}	15.5 ± 0.63^{Aa}
40	5.79 ± 0.19^{Ac}	12.4 ± 0.12^{Ab}	16.2 ± 0.92^{Aa}
45	5.50 ± 0.30^{Ac}	11.7 ± 0.38^{Ab}	13.4 ± 0.43^{Ba}

Table 3.1. Moisture contents (%) of EWP-GLY-water mixtures with GLY contents of 0, 35, 40, and 45 % and water activities (a_w) of 0.34, 0.48 and 0.64 at 23.1 °C.
*Different capital case letter indicate significant difference ($P \leq 0.05$) between EWP mixtures with different glycerol contents and different small case letter indicate significant difference ($P \leq 0.05$) between EWP mixtures conditioned to different water activity.
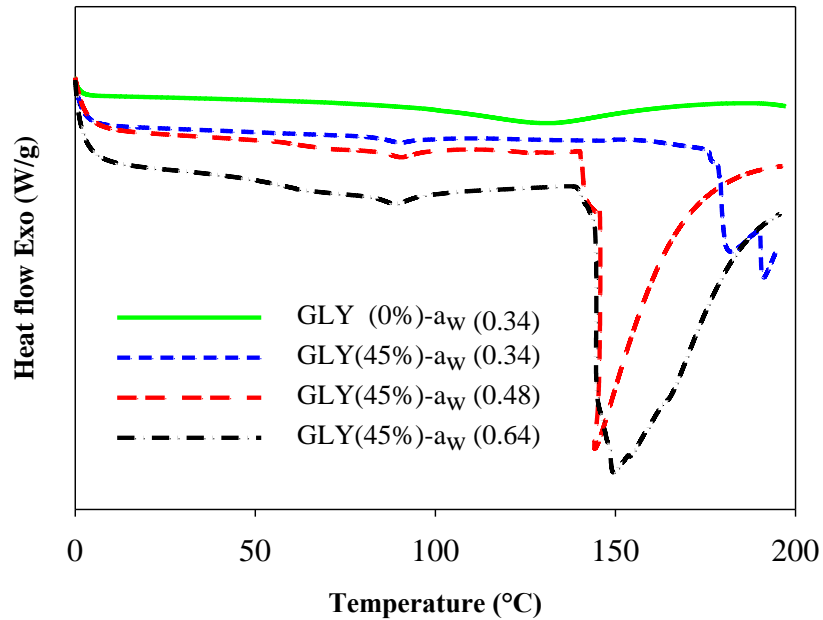
Figure 3.1. DSC thermograms of EWP-GLY-water mixtures with different GLY content (0 and 45 %) and water activities (0.34, 0.48, and 0.64). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of the thesis.

GLY (%)	Second-order transition temperatures (°C)					
	First			Second		
	a_w of 0.34	a_w of 0.48	a_w of 0.64	a_w of 0.34	a_w of 0.48	a_w of 0.64
0	116.2 ± 0.28^{Aa}	114.1 ± 0.38^{Ab}	113.0 ± 0.11^{Ac}	na	na	na
35	89.3 ± 0.20^{Ba}	88.3 ± 0.03^{Bb}	87.5 ± 0.07^{Bc}	155.9 ± 4.13^{Aa}	143.5 ± 3.12^{Aab}	132.2 ± 7.29^{Bb}
40	88.1 ± 0.18^{Ca}	87.6 ± 0.19^{Cb}	85.9 ± 0.07^{Cc}	166.5 ± 3.68^{Aa}	151.1 ± 6.28^{Ab}	162.1 ± 1.55^{Aa}
45	86.8 ± 0.11^{Db}	87.7 ± 0.17^{Ca}	85.7 ± 0.21^{Cc}	169.9 ± 12.9^{Aa}	140.5 ± 5.17^{Ab}	150.5 ± 5.95^{Aab}

Table 3.2. First and second second-order transition temperatures (°C) of EWP-GLY-water mixtures with GLY contents of 0, 35, 40, and 45 % and water activities (a_w) of 0.34, 0.48 and 0.64 at 23.1 °C.
*Different capital case letter indicate significant difference ($P \leq 0.05$) between EWP mixtures with different glycerol contents and different small case letter indicate significant difference ($P \leq 0.05$) between EWP mixtures conditioned to different water activity.
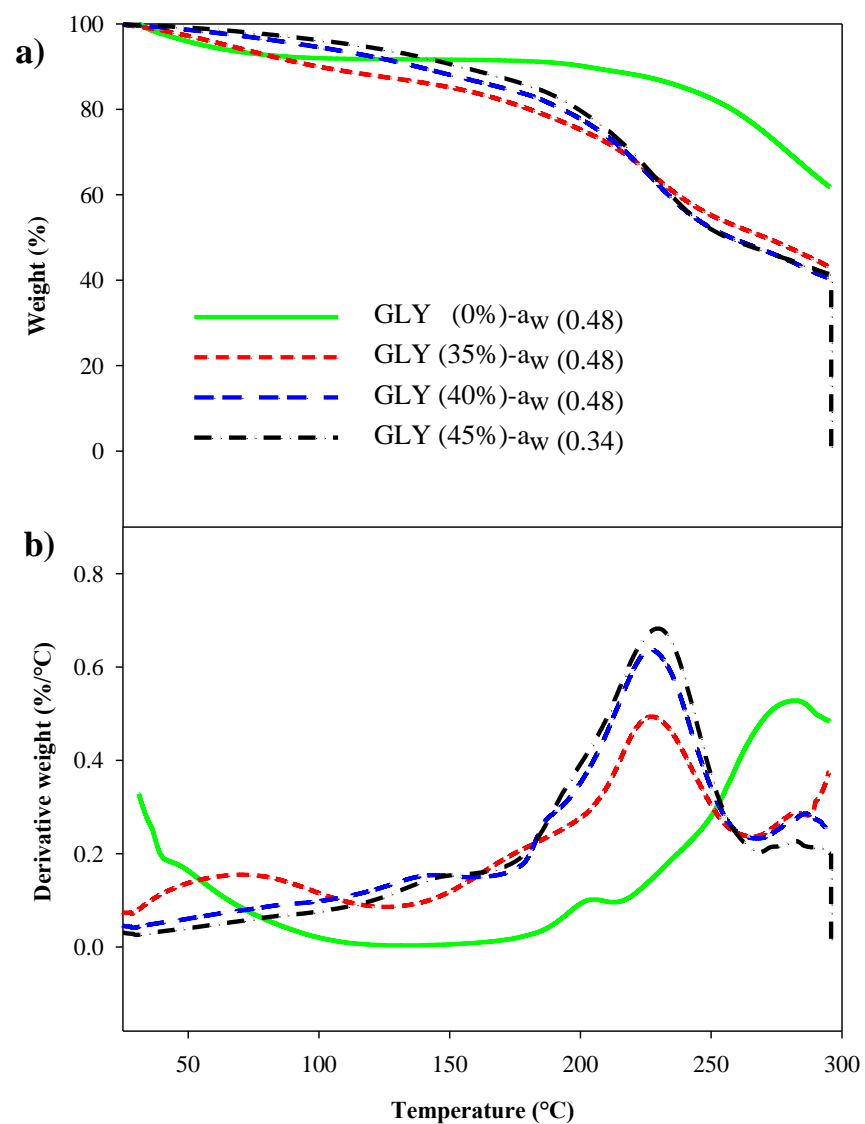
Figure 3.2. Weight change (a) and derivative weight change (b) of EWP-GLY-water mixtures with different GLY content (0, 35, 40 and 45 %) and water activities (0.34 and 0.48)

Figure 3.3. Photographs of the EWP-GLY-water mixtures with GLY contents of a) 35 %, b) 40 %, and c) 45 % and 0.48 water activity (area of 2 cm x 1.5 cm).
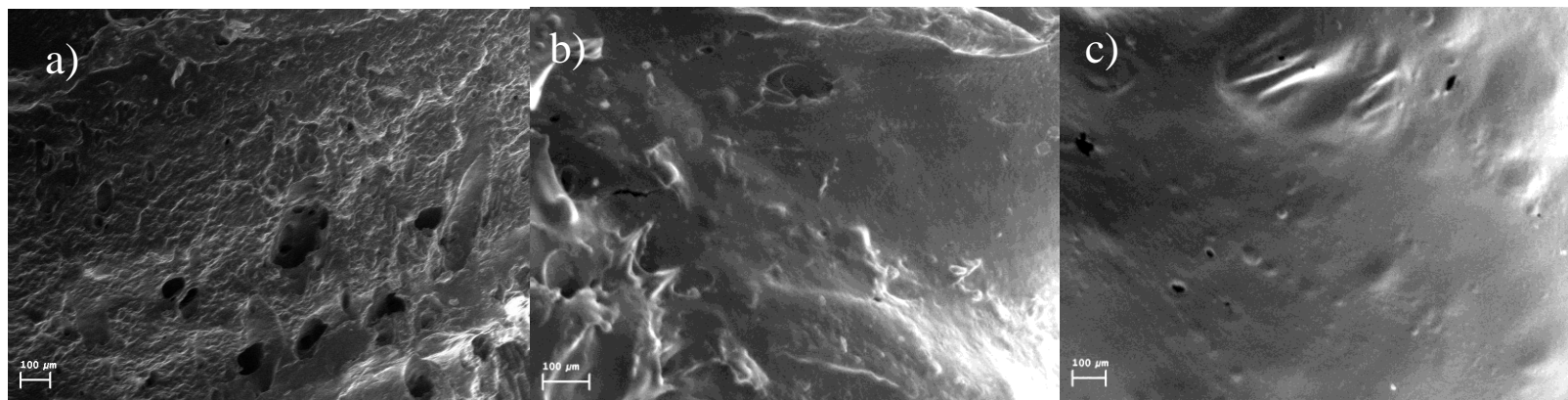
Figure 3.4. VP-SEM micrographs of the cross sections of GLY-EWP-water mixtures with GLY contents of a) 35 %, b) 40 %, and c) 45 % and 0.48 water activity (200x magnification; 1200 μm x 1600 μm (height x width)).

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CHAPTER 4

INFLUENCE OF GLYCEROL AND WATER ACTIVITY ON THE PHYSICAL, MECHANICAL AND MORPHOLOGICAL PROPERTIES OF COMPRESSED EGG WHITE-BASED BIOPLASTICS

Abstract

In this paper, the effects of glycerol (35, 40 and 45%) and water activity (0.34 and 0.48) on the physical, mechanical and morphological properties of compressed egg white-based bioplastics are investigated. Lighter, more reddish and less yellowish sheets with a decreased thickness and second-order transition temperature, improved mechanical properties (increased flexibility and decreased rigidity and stiffness) and constant moisture content can be obtained by increasing water activity. Increasing the GLY content results in the same type of changes but to a lesser extent and increased moisture content. Increasing both, water activity and GLY content, leads to a more pronounced effect in some of these properties. This study demonstrates that compressed egg white-based bioplastics with desired properties can be obtained by adjusting water activity and GLY content.

Keywords: egg white protein, bioplastic, water activity, glycerol, compression, properties

4.1. Introduction

Government, industry, and consumers are more and more interested in ways to reduce the municipal solid waste resulting from plastics and to decrease the dependence of the production of plastics on petrochemical sources (Kim, 2008; Koutsimanis et al., 2012; Mohanty and Misra, 2002). These desires have led to the intensification of research to develop plastics that are biodegradable and made from renewable resources. These plastics are currently known as bioplastics (Stevens 2002).

Proteins offer great potential for the production of bioplastics (Gonzalez-Gutierrez et al., 2010; Guerrero et al., 2011). Proteins are molecules comprised of hundreds of amino acids with the capability of forming both weak and strong linkages and can result in a three-dimensional network stabilized by low-energy interactions and strengthened by covalent bonds (Pommet et al., 2003). Protein-bioplastics can be obtained by direct mixing of the proteins with a plasticizer under low moisture conditions, and posterior exposure to heat and pressure using compression or extrusion processes (Hernandez-Izquierdo and Krochta, 2008a). Plasticizers are incorporated to modify the three-dimensional structure of the proteins through the reduction of intermolecular forces which facilitates the movements of the protein chains relative to each other and thereby eases processability (di Gioia and Guilbert, 1999). Plasticizer can also reduce protein decomposition during processing by decreasing the transition temperatures through increasing the free volume of the protein network (Lee et al., 2012). In addition, plasticizers promote the flexibility and reduce the brittleness of the resulting film or sheet (Sothornvit et al., 2007; Cunningham and Ogale, 2000).

Water and glycerol are two well-known plasticizers for proteins (Pommet et al., 2005; Slade & Levine 1993; Sobral & Habitante, 2002; Sothornvit et al., 2007). Their efficiency results from their small size which allows them to easily interpose themselves between the protein chains and to decrease the forces holding the chains together (di Gioia & Guilbert, 1999). Although water and glycerol are needed to improve protein matrix processability and to overcome film/sheet brittleness, their amount greatly can affect the properties of the resulting film or sheet. Differences in mechanical properties, color, thermal behavior, and morphology in extruded and compressed protein-based sheets and films have been attributed to differences in water and glycerol contents (Hernandez-Izquierdo et al., 2008b; Sothornvit et al., 2007; Zhang et al., 2001). Therefore, the understanding of the impact of the plasticizer type and content on the properties of the protein-based bioplastic is necessary in order to improve the properties and suitability of these plastics for specific applications. In addition, this understanding can create bioplastics with desired properties.

Recent studies have shown the feasibility to produce bioplastics from egg white proteins (Jerez et al, 2007; Lee et al., 2012). Egg white-based bioplastic have been reported to be easily processed at low temperatures (Jerez et al., 2007). Sheets of egg white-based bioplastic have been manufactured by thermo-mechanical compression and have been described as highly transparent and highly elastic (Gonzalez-Gutierrez et al., 2010). Given these advantages and that egg protein meets food grade standards (Kreider, 2012), this new bioplastic could be considered as a feasible material for biodegradable packaging and other plastic products. To date, only a few studies have focused on egg white-based bioplastics (Jerez et al., 2007; Gonzalez-Gutierrez et al., 2010; Gonzalez-Gutierrez et al., 2011). These

studies are limited to one glycerol and water activity and therefore, there is no systematic information about the effects of glycerol and water activity contents on the properties of egg white-based bioplastics in the literature. Our study's goal was to understand the role of both, glycerol and water activity contents, and of their interaction, on the properties of sheets made of egg white-based bioplastics. Mechanical and thermo-mechanical properties, morphology, optical properties, and thermal behavior of these bioplastic sheets have been investigated. Understanding these varying properties bring us one step closer to useful applications of sheets and film made from this new bioplastic.

4.2. Materials and methods

4.2.1 Materials

Desugared, spray dried egg white protein (EWP) powder (minimum 92% solids, minimum 80% protein) was obtained from Rose Acre Inc. (Seymour, IN, United States). Glycerol (GLY) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO, Unites States). Magnesium chloride ($MgCl_2$) and sodium nitrate ($NaNO_3$) were supplied by Columbus Chemical Industries Inc. (Columbus, WI, United States). Standard saturated salt solutions of 0.30 ± 0.01 and 0.50 ± 0.01 water activities were purchased from Decagon (Decagon Devices Inc, Pullman, WA, USA).

4.2.2 Methods

4.2.2.1 Preparation of EWP sheets

2.2.1.1. Egg white-based mixtures preparation: EWP-GLY-water mixtures with GLY contents of 35, 40 and 45 % on dry basis (w/w) were prepared by intensive mixing of EWP and GLY for 10 minutes using a mortar and pestle, and posterior pre-conditioning to water activities of 0.34 ± 0.01 and 0.48 ± 0.02 at 23.1 ± 0.1 °C. The pre-conditioning was performed by placing the mixtures on aluminum dishes and then storing these for three days in closed environments maintained at 34 % and 55 % RH. A closed storage container containing a saturated solution of $MgCl_2$ was used to condition the mixtures to 34 % RH (0.34 ± 0.01 water activity). A room conditioned at 55 ± 5% RH was used to condition the mixtures to 0.48 ± 0.02 water activity. Three replicates were prepared per GLY content and per pre-conditioning condition. The water activity of the mixtures was verified using an AquaLab model CX-1 (Decagon Devices Inc, Pullman, WA, USA). The instrument was allowed to warm up for one hour and then calibrated with standard saturated salt solutions of 0.30 ± 0.01 and 0.50 ± 0.01 water.

2.2.1.2. Egg white-based sheet preparation: Egg white-based mixtures resulting from combinations of 35, 40 and 45 % GLY and 0.34 and 0.48 water activity were converted into sheets by thermo-mechanical procedure using a compression machine, Model-M, Carver Laboratory Press (Carver Inc., Wabash, IN, USA). EWP-GLY-water mixtures were compressed at 40 °C above the average first second-order transition temperature obtained for the EWP-GLY mixtures according to Lee et al. (2012), with a residence time of 8 min, and a pressure of 2.0 MPa. Mixtures were placed between aluminum foil sheets while pressing to avoid the molten material to stick to the metal plates. Resulting samples were cut into 5 x 10 cm strips and then repressed under the above

reported conditions to get EWP sheets with a thickness close to that of current commercial sheets. Each of the EWP-GLY-water mixtures was prepared three times to generate three replicates. EWP sheets were obtained from the replicates and conditioned at 55 % RH and 23 $^{\circ}$C for 3 days prior to use.

4.2.2.2 Moisture content determination

The moisture content of the EWP sheets was determined according to AOAC Intl. (2006) (method 937.01). Weight measurements were taken after 24 h by removing the samples from a vacuum oven (70 $^{\circ}$C and 50 mmHg vacuum), and equilibrating these to room temperature inside a desiccator. Three different EWP sheets per type of sheet were tested.

4.2.2.3 Thickness determination

The thickness of EWP sheets was measured at five random positions using an electronic digital micrometer (Fowler® 0-1" Digital Counter Micrometer, Port Washington, NY, USA). A mean thickness from three different sheets was calculated.

4.2.2.4 Thermal characterization

4.2.2.4.1 Differential Scanning Calorimetry

The second-order transition temperature of the EWP sheets was determined by using a differential scanning calorimeter (DSC Q100; TA Instruments, Newcastle, DE) with a liquid nitrogen cooling system. An amount between 7 to 10 mg of each type of sheet was hermetically sealed in an aluminum pan (TA Instruments, Newcastle, DE, USA),

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equilibrated to 0 °C, and then heated to 200 °C at a rate of 20 °C/min. During each run, the DSC cell was flushed with nitrogen at 70 ml/min to maintain an inert environment. The instrument was calibrated using pure indium. TA analysis software was used for data analysis in accordance with ASTM D3418 (ASTM, 2008). One sample of three different EWP sheets per type of sheet was tested.

4.2.2.4.2 Thermogravimetric analyses

The decomposition temperature of the EWP sheets was determined by using a thermogravimetric analyzer (TGA Q50; TA Instruments, Newcastle, DE, USA). An amount between 7 to 10 mg of EWP sheet was placed in an aluminum pan (TA Instruments, Newcastle, DE, USA) and then heated from 25 to 300 °C at a rate of 20 °C/min. The percentage of weight loss of each sample as a function of temperature under a nitrogen-air (40 % - 60 %) atmosphere was analyzed. Samples from three different EWP sheets were tested.

4.2.2.5 Mechanical characterization

4.2.2.5.1 Dynamic Mechanical Analyses

A dynamic mechanical analyzer (DMA Q800; TA instruments, New Castle, DE, USA) was used to measure the thermo-mechanical properties of the EWP sheets. The sheets were cut into rectangular specimens of 1.6 x 0.5 cm and then tested using the tension mode, frequency 1 Hz and amplitude 20 μm, over a temperature range of 25 to 150 °C at a heating rate of 5 °C/min under the DMA-multi-frequency-strain operational mode in accordance with ASTM D4065 (ASTM, 2006). The storage modulus (G') and tan delta (tan δ) were determined. One sample of three different EWP sheets per type of sheet was tested.

4.2.2.5.2 Tensile analyses

EWP sheets were cut into several rectangular pieces of a size 10 x 1.0 cm each. The elongation at break (E_b), tensile strength (σ_{max}), and modulus of elasticity (E) of each piece were measured according the ASTM D882 (ASTM, 2010) using an Instron Universal Testing Machine UTS SFM – 20 (United Calibration Corporation, Huntington Beach, CA, USA) with a load cell of 1000 lb. A speed of 20 in/min and an initial grip separation of 2 in were used as the elongation at break was greater than 100 %. A minimum of five pieces obtained from three different EWP sheets of each type of sheet was tested.

4.2.2.6 Optical characterization

4.2.2.6.1 Color

The color of EWP sheets was measured using a Labscan XE colorimeter (Hunter Laboratories, Reston, VA, USA) and characterized by the CIE L*a*b* system. This system can be visualized as a cylindrical coordinate system in which the axis of the cylinder is the lightness variable L*, ranging from 0 to 100%, and the radii are the 49 chromaticity variables a* and b*, where variable a* ranges from green (negative) to red (positive) and variable b* ranges from blue (negative) to yellow (positive). Nine measurements of three different EWP sheets of each type of sheet were taken.

4.2.2.6.2 Transmittance

EWP sheets were cut into rectangular pieces of 3.0 x 6.0 cm. The transmittance (%) of each piece was measured using a spectrophotometer (Lambda 25 UV/VIS Spectrometer; PerkinElmer Instruments, Waltham, MA, USA) in the spectral range from 300 to 850 nm

and with a scan speed of 480 nm per minute. This spectrometer has a specialized attachment that reassembles the refracted light that passes through the film, thus giving a more accurate summary of the total light transmitted. The transmittance values obtained at a wavelength of 600 nm are reported. Three different EWP sheets per type of sheet were tested.

4.2.2.7 Morphological characterization

The morphology of the EWP sheets was observed using a Carl Zeiss Variable Pressure Scanning Electron Microscope (VP-SEM) EVO LS25 (Carl Zeiss Inc., Thornwood, NY, USA) equipped with a LaB6 gun. Non-coated EWP sheets were frozen using liquid nitrogen and cracked off with cutting pliers. Micrographs of the surfaces and cross sections of the pieces were obtained by using beam energy of 20 kV. Micrographs were taken at 50x magnification and represent a surface of 5600 μm x 4200 μm for the surface area and at 350x magnification and represent a surface of 860 μm x 640 μm for the cross sectional area. VP-SEM was used instead of high pressure SEM in order to prevent GLY from leaking due to the high pressure and to minimize damage caused by charging and coating. Thus, the mixtures were kept as close to original as possible.

4.2.2.8 Statistical analysis

A two-factor completely-randomized experimental design was used to study the effect of glycerol content (35, 40 and 45 %) and water activity (0.34 ± 0.01 and 0.48 ± 0.02) on the thermal, mechanical, and optical properties, moisture content, and thickness of the EWP sheets. The statistical software Minitab 15 (Minitab Inc, State College, PA, USA)

was used to perform a one-way analysis of variance (ANOVA; Tukey test; $p \leq 0.05$). Three different EWP-GLY sheets per type of mixture were tested.

4.3. Results and Discussion

EWP sheets were developed based on the characterization of EWP-based mixtures according to Lee et al. (2012). Thus, the EWP mixtures were pre-conditioned using water activities of 0.34 ± 0.01 and 0.48 ± 0.02 and were compressed into sheets at a temperature of 130 °C (40 °C above the average first second-order transition temperature obtained for the EWP mixtures). In the following, the characterization of the resulting EWP sheets is presented.

4.3.1 Moisture content of EWP sheets

Table 4.1 lists the moisture contents (%) of EWP sheets with different GLY contents (35, 40 and 45 %), pre-conditioned to water activities of 0.34 ± 0.01 and 0.48 ± 0.02 at 23.1 ± 0.1 °C, and post-conditioned at 55 % RH and 23.1 ± 0.1 °C. As observed, the moisture contents of EWP sheets ranged between 13.8 and 15.6 %. These values are higher than those reported for the corresponding EWP mixtures (Lee et al., 2012). This increase in moisture resulted from the post-conditioning process and shows the capability of EWP sheet to absorb water from the environment. The moisture content of EWP sheets was the same ($p > 0.05$) for both values of water activity used in the pre-conditioning, and thus seems to be independent of the pre-conditioning water activity. However, the moisture content of EWP sheets differed between GLY contents. EWP sheets containing 35 % of GLY had less moisture than those containing 40 and 45 % of GLY. This could be attributed

to the hygroscopic character of the GLY (resulting from the three hydroxyl groups in its structure), which allowed drawing of additional water into the EWP network. While no significant differences between the moisture contents of EWP sheets with 40 and 45 % GLY and pre-conditioned at either 0.34 or 0.48 water activity could be identified, the trend (Figure 4.1) seems to indicate that higher GLY lead to higher moisture contents. This trend is in agreement with the results of Coupland et al. (2000) who studied the effect of GLY on the ability of WPI films to absorb water through modeling moisture sorption isotherms and reported that the higher the glycerol content in the film the higher the amount of water absorbed by this (Coupland et al., 2000). However, Hernandez-Izquierdo et al. (2008b) did not find significant differences in moisture contents among extruded whey protein sheets with different GLY contents (45.8, 48.8, and 51.9 %). Studies exposing EWP sheets to higher RH would need to be conducted in order to determine whether 15% is the maximum amount of water that EWP sheets can absorb or not at the GLY contents tested.

4.3.2 Thickness of EWP sheets

Table 4.1 lists the thicknesses (mm) of the EWP sheets under investigation and described above. The thickness of EWP sheets varied with the amount of GLY and the water activity in EWP-GLY mixtures, and it ranged from 0.49 to 1.13 mm. The water activity to which the EWP-GLY mixtures were pre-conditioned had a larger effect than the GLY content. EWP sheets resulting from mixtures pre-conditioned to 0.48 water activity were 28 to 46 % thinner than those pre-conditioned to 0.34 water activity. The range of thickness differences of 28 to 46 % was caused by the different GLY contents. EWP sheets made from mixtures with GLY contents of 35 and 40 % were about 30 % thinner when the

mixtures were pre-conditioned to 0.48 instead of 0.34 water activity while this difference was more pronounced (46 %) in the case of a 45 % GLY content. In addition, the differences in thickness of EWP sheets with different GLY contents were larger for the mixtures pre-conditioned to 0.48 water activity than for those pre-conditioned to 0.34 water activity. These results suggest that there is a combined effect between water activity and GLY content. The interactions of the water and of the GLY with the EWP increase the free volume in the matrix. This allowed the realignment of the protein matrix and consequently its compactness during the pressing process which led to thickness reduction. All these results also suggest that EWP sheets with desired thicknesses can be easily tailored by choosing a specific water activity and GLY content. The effect of the GLY content on the sheet thickness has also been observed for WPI sheets (Sothornvit et al., 2007). The average thicknesses of WPI sheets were reported as 1.32 ± 0.27, 0.92 ± 0.21 and 0.57 ± 0.10 mm for 30, 40 and 50 % GLY, respectively, but not discussed. No effect of water activity was studied. In another study, these researchers reported that WPI films made of WPI and water were thinner than those made of WPI and GLY (0.687 ± 0.09 and 0.785 ± 0.32 mm, respectively) for the same water or GLY content in the total mixture weight (Sothornvit et al., 2003).

4.3.3 Optical properties of EWP sheets

Table 4.2 presents the color properties of the EWP sheets under investigation and described above. The lightness (L*) and color (a* and b*) of the EWP sheets were affected ($p \leq 0.05$) by both, water activity and GLY content, with the former having a greater effect. EWP sheets made from mixtures pre-conditioned to 0.48 water activity were lighter, more

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reddish and less yellowish than the EWP sheets made from mixtures pre-conditioned to 0.34 water activity. The GLY content affected the a* values of all EWP sheets but affected the L* and b* values only of EWP sheets made from mixtures pre-conditioned to 0.48 water activity. EWP sheets made from mixtures pre-conditioned to 0.48 water activity became lighter ($p \leq 0.05$) as L* increased from 87.0 to 88.7, more reddish ($p \leq 0.05$) as a* increased from -2.27 to -1.88, and less yellowish ($p \leq 0.05$) as b* decreased from 15.2 to 9.55 with the increase of GLY. Hydrolysis during processing may have rendered amino group ($-NH_2$), which might undergo interaction with carbonyl group via Maillard reaction in the egg white proteins as reported for gelatin by Hoque et al. (2011). This could be the reason of the increased red color of the EWP sheets in the presence of a higher content of water. Since the moisture content of the EWP sheets was increased with the glycerol content, the more the glycerol present the more the red color of the EWP sheets. In agreement with the decreased b* value of the EWP sheets with the increase of GLY, Hernandez-Izquierdo et al. (2008b) reported that the b* value of extruded WPI sheets decreased as the GLY content increased (Hernandez-Izquierdo et al., 2008b). On the contrary, Gennadios et al. (1996) and Vanin et al. (2005) reported that neither plasticizer type (glycerol, polyethylene glycol or sorbitol) nor concentration had an effect on the color films made from egg albumen and gelatin, respectively. This might be because their films are thinner than our sheets (0.13mm vs 0.5-0.8mm) and therefore, the change of color in their films was insignificant.

Since the GLY content affected significantly ($p \leq 0.05$) the thickness of the EWP sheets (from 0.76 to 0.49 mm), a complementary experiment was performed in order to

determine the impact of the thickness on the color of the EWP sheets. The experiment consisted of measuring and comparing the color of EWP sheets after a different number of presses. Figure 4.2 presents the color results of EWP sheets made of mixtures with 40 % GLY and pre-conditioned to a water activity of 0.48 ± 0.02 at 23.1 ± 0.1 °C, and post-conditioned at 55 % RH and 23.1 ± 0.1 °C after their 1st, 2nd and 3rd press. The thickness of the sheets was affected by the number of times the material was pressed (1st press: 1.09 mm, 2nd press: 0.64 mm, and 3rd press: 0.51 mm) and had a significant ($p \leq 0.05$) effect on the color of the EWP sheets. The EWP sheets became significantly ($p \leq 0.05$) lighter as L* increased from 85.2 to 87.7, less yellow as b* decreased from 21.0 to 12.8 and less green as a* increased from -2.76 to -2.11 as the thickness of the sheets decreased. These results are supported by Gennadios and others who noted that the color of a protein-based film can be affected by its moisture content and thickness. These authors reported that thicker soy-based films were more yellowish (higher positive b* values) (Gennadios et al., 1996). The number of pressings could have had an indirect effect on the color of the EWP sheets. Pressing results in protein alignment as reported by Jerez et al. (2007) and this alignment could have assisted in reducing its thickness and consequently, resulting in changes in color of the EWP sheets.

Table 4.2 presents the transmittance values (%) of a subset of the EWP sheets described above (only those pre-conditioned to 0.48 ± 0.02 water activity). EWP sheets are a highly-transparent plastic with a transmittance of around 89%. One would expect them to be opaque as albumin turns opaque after thermal treatment. However, the random aggregates produced by the heat-denatured ovalbumin which cause the opaqueness can be

dissociated by the application of pressure during thermo-mechanical processing as shown by Jerez et al. (2007) using Atomic Force Microscopy. No significant differences (p > 0.05) were found across sheets with different GLY content. The EWP sheets produced in this study were more transparent than those reported by Gonzalez-Gutierrez et al. (2010) (89% vs 38%). The different transparency may be due to the difference in thickness of the samples (275 to 500%; 0.49 to 0.79 vs 3 mm).

4.3.4 Thermal characterization of EWP sheets

A dynamic mechanical characterization was performed on a subset of the EWP sheets described above (only those pre-conditioned to 0.48 ± 0.02 water activity). The storage modulus (G') and tan delta (tan δ) of these EWP sheets are presented as a function of temperature in Figure 4.3. The peak observed in the tan δ curve (Figure 4.3a) is indicative of chain motion. However, this peak is neither sharp nor intense which denotes little chain motion. This was expected since the peak most likely represents the alteration of the three-dimensional structure of the proteins resulting from the breakage of their stabilizing bonds (transition from folded proteins to unfolded proteins) and therefore, not much chain mobility can be expected. The glass transition temperature (Tg) of EWP sheets was obtained from the maximum peak values of the tan δ curve and was between 82 - 85 °C. This second-order transition temperature matches the one reported for EWP-based mixtures and solutions and that has been attributed to the denaturation of the ovoalbumin (Lee et al., 2012; Van der Placken et al., 2005). This second-order transition temperature also matches the maximum in tan δ occurring at around 80 °C observed by Jerez et al. (2007) for EWP-based sheets with 37.5 % of GLY. This Tg is a little bit higher than that

reported for other protein-based sheet containing the same GLY content and also obtained by thermo-mechanical processing. Ogale and others reported a Tg of 70.9 °C for soy protein isolate (SPI)-40 % GLY films (Ogale and Cunningham, 2000) and Jerez and others observed a Tg of 60°C for wheat gluten (WG)-50 % GLY (Jerez et al., 2007). The higher Tg of the EWP sheets could be explained by EWP having different amino acid groups with stronger interactions which are harder to break and consequently require more vibrational (thermal) energy to change the EWP matrix from the glassy state to the rubbery state. In addition, GLY could have had some effect too. GLY may be able to mix better with SPI and WG than with EWP. The increase in GLY content decreased the Tg of the EWP sheets significantly ($p \leq 0.05$) as observed by the shifting of the peak in the tan δ curve towards lower values with increasing the GLY content (85.0 ± 0.2, 83.4 ± 0.6, and 80.8 ± 0.2 °C for 35, 40 and 45 % of GLY, respectively). The GLY content also affected the height of the peak in the tan δ curve. Its height increased with the GLY content, confirming the increased movement of the polymer chains when increased the amount of GLY. However, no significant differences ($p > 0.05$) were observed for EWP sheets with high GLY content. In agreement, Lee et al. (2012) did not find significant differences between the first second-order transition temperatures of EWP-GLY-water mixtures containing 40 and 45 % GLY. A decrease of Tg with the increase of GLY and/or other plasticizer content has been reported for other protein-based films. Sobral and others observed that the Tg of muscle protein-based films decreased from 1.85 to -63.2 °C with an increase of GLY content from 15 to 65 % (Sobral et al., 2005). Vanin et al. (2005) reported that an increase of GLY content from 10 to 25 % decreased the Tg of gelatin-based films from 82.5 to 35.5 °C. However, an increase of Tg from 35.5 to 41.4 °C was observed at higher GLY content (25

and 30% GLY) although this was not significant ($p > 0.05$). These authors also observed the same reduction when using other plasticizers such as propylene glycol (PG), ethylene glycol (EG) and diethylene glycol (DEG), (from 69.9 to 61.9, from 98.5 to 39.9 and from 45.8 to 13.4 °C for 10 vs. 30 % plasticizer content (PG, EG, and DEG, respectively)) (Vanin et al., 2005).

Figure 4.3b shows the decrease of G' of EWP sheets with the increase of GLY content. G' changed from 210 MPa to 130 MPa and to 100 MPa with the increase of GLY content in the sheet from 35 to 40 and to 45 %, respectively. This decrease in G' with the increase of GLY indicates that more energy and stress is needed to break up the interactions of the polymer network in sheets containing 35 % GLY than in those containing 40 and 45% GLY. The increase in GLY in the EWP sheets most possibly weakened the interactions between the protein chains. The mobility of the polymer chains seems to be less restrained with the increase of GLY. These results are in agreement with those reported by Ogale and Cunningham (2000) who found that SPI films with 20 % GLY had higher G' values than SPI films with 30 and 40 % GLY.

Figure 4.4a presents the DSC thermograms of the different EWP sheets under study. The thermograms show second-order transition temperatures of 150 °C and higher for EWP sheets. The second-order transition obtained at 82 °C when using the DMA technique was not denoted. This might be due to different sensitivities of the DMA and DSC techniques. Menard (1999) reported that the DMA technique is 10 to 100 times more sensitive to changes of the Tg than the DSC technique (Menard, 1999). The EWP sheets were completely amorphous as shown by the absence of crystalline peaks and therefore, the second-order transition observed at 150 °C and above can be defined as a high-temperature

second-order transition or a second second-order transition temperature instead of as a melting temperature. Ogale and Cunningham (2000) also found a second second-order transition temperature in SPI films obtained by thermo-mechanical processing. This was associated with local regions of more highly concentrated protein or with a loss of plasticizer. This was GLY dependent as 119 and 150 °C were obtained for GLY contents of 30 and 40 %, respectively (Ogale and Cunningham, 2000). In contrast, the second second-order transition temperature of EWP sheets decreased as the glycerol content increased from 35 to 45 %. Table 4.1 lists the second second-order transition temperatures of the EWP sheets, ranging from 150 to 177 °C. The water activity to which the EWP-GLY mixtures were pre-conditioned affected the second second-order transition temperature of EWP sheets significantly ($p \leq 0.05$) except for the case with low GLY content (35 %). Higher water activities during pre-conditioning lead to significantly smaller second second-order transition temperatures ($p \leq 0.05$): 159.6 and 152.8 °C (water activity of 0.48) instead of 174.1 and 169.2 °C (water activity of 0.34) for 40 and 45 % GLY content, respectively. Second second-order transition temperatures of EWP sheets were also affected by the GLY content (35, 40 and 45 %) but only for EWP sheets made from EWP-GLY-moisture mixtures pre-conditioned to 0.48 water activity. This suggests that EWP-GLY mixtures pre-conditioned with water activities higher than 0.34 lead to a better thermoplasticization. This might be explained by the increased free volume along the polymer matrix caused by the addition of water which enables more chain movement.

Figure 4.4b shows the TGA thermograms of EWP sheets. EWP sheets with 40% GLY and pre-conditioned at water activity of 0.48 were used to understand the thermal stability including the thermal degradation of EWP sheets in general. The weight loss of

EWP sheets is relatively small until 190 °C, moderate between 190 and 250 °C and significant above 250 °C. The degradation temperature of EWP sheets (190 °C) is higher than that reported for EWP-GLY-water mixtures (170 °C) (Lee et al., 2012). This shows that the thermal compaction during the compression process produces a more stable structure of the EWP matrix.

Summarizing the thermal results, second second-order transition temperature of EWP sheets can be lowered with an optimum combination of water activity for pre-conditioning and GLY concentration, and decomposition temperature of the EWP matrix can be increased with thermal compaction and temperature modification. These results suggest that we can adjust the formulations of the EWP sheets to better thermoplasticize the EWP.

4.3.5 Mechanical characterization of EWP sheets

In order to characterize the impact of the composition (amount of GLY and water activity) of the EWP sheets on their mechanical properties, several tensile properties were determined for EWP sheets with different glycerol contents (35, 40 and 45 %) and pre-conditioning conditions (water activities of 0.34 ± 0.01 and 0.48 ± 0.02). The flexibility and stretchability (E_b), the maximum tensile stress sustainable before breakage (σ_{max}), and the rigidity and stiffness (E) of the EWP sheets were measured in the machine direction. The results are presented in Figure 4.5. EWP sheets had an E_b ranging between 86.2 and 154.0 %, an E ranging between 13.4 and 36.7 MPa, and a σ_{max} ranging between 6.6 and 9.7 MPa.

In agreement, Gonzalez-Gutierrez et al. (2010) reported an E_b of 130.4 and a σ_{max} of 7.1 for EWP sheets with 37.5 % of glycerol and 0.48 water activity (Gonzalez-Gutierrez et al., 2010). The E_b and E of the EWP sheets were greatly affected by both the amount of GLY and the water activity to which EWP-GLY mixtures were pre-conditioned while the σ_{max} of the EWP sheets was only affected by the GLY content.

The water activity to which the EWP-GLY mixtures were pre-conditioned had a similar effect to that of the GLY content on the E_b of the EWP sheets (Figure 4.5a). The increase of water activity from 0.34 to 0.48 significantly ($p \leq 0.05$) increased the E_b of the EWP sheets by 25 to 42 % (depending on the GLY content), and the increase of GLY content from 35 to 45 % significantly ($p \leq 0.05$) increased the E_b of the EWP sheets by 36 and 43 % for 0.34 and 0.48 water activity, respectively. Therefore, an increase in water activity and/or GLY content results in more flexible EWP sheets. As mentioned earlier, GLY and water act as plasticizers and increase the free volume of the matrix, thereby allowing the structure to increase in stretchability due to the greater alignment of the protein chains during pressing. This increase evidences the compatibility of the protein with the water molecules and the GLY. Sothornvit et al. (2007) reported an E_b for compressed WPI sheets between 43 to 94 % for GLY contents between 30 and 50 %. Therefore, compressed WPI sheets are much more rigid than EWP sheets (E_b of 86 to 154 %). Compressed EWP sheets are also more flexible than compressed soy protein isolate (SPI) sheets since an E_b of 74.5 % has been measured for these sheets (Cunningham et al., 2000).

However, compressed EWP sheets have a similar flexibility to that of wheat gluten (E_b 121 %; Gallstedt et al. 2004).

The E of the EWP sheets was reduced for both, GLY content and water activity. Significant reductions ($p \leq 0.05$) of 28 to 49 % across water activities and of 29 to 49 % across GLY contents for 0.34 and 0.48 water activities, respectively, were observed (Figure 4.5c). Changes on GLY content affected E more at lower water activity. However, the higher the water activity the lower the E for the same GLY content. An increase in water activity from 0.34 to 0.48 resulted in a reduction of 49 % (36.7 to 18.8 MPa), 40 % (26.4 to 15.9 MPa), and 28 % (18.6 to 13.4 MPa) of the E of EWP sheets containing 35, 40 and 45 % GLY, respectively. Therefore, E seems to be reduced with the increase of both, water activity or GLY content. This may be due to the higher degree of plasticization EWP caused by higher GLY and water contents. As mentioned earlier, GLY and water act as plasticizers and increase the free volume of the matrix. This allowed the realignment of the protein matrix and consequently its compactness during the pressing process. thereby allowing this to be more compact. Therefore, the increase in both, GLY and water activity, can make EWP sheets less rigid and stiff. Compared to other protein-based sheets, EWP sheets are less elastic than WPI sheets but more elastic than WG sheet (E of 16, 144, and 3 MPa, respectively) (Hernandez-Izquierdo et al., 2007; Gallstedt et al., 2004).

The σ_{max} of EWP sheets decreased significantly ($p \leq 0.05$) with the increase of GLY but not ($p > 0.05$) with that of the water activity (Figure 4.5b). As the GLY content increased from 35 to 45 %, a reduction of 31 % and of 22 % in σ_{max} was observed for water activates of 0.34 and 0.48, respectively. Therefore, stronger or weaker EWP sheets

can be obtained by changing the GLY content. This effect could be because GLY as a plasticizer might have reduced the inter- and intra-molecular forces holding the EWP together to such an extent that less stress is required to break the EWP sheets. σ_{max} for compressed SPI and WG with 40 % GLY content have been reported to be 2.6 and 1.9 MPa, respectively (Cunningham et al., 2000; Gallstedt et al., 2004). Therefore, EWP sheets are stronger (σ_{max} 7.6 MPa) than SPI and WG sheets. However, they have strength similar to that of WPI sheets (σ_{max} 8.0 MPa) (Hernandez-Izquierdo 2007).

4.3.6 Morphology of EWP sheets

Figure 4.6 shows the VP-SEM micrographs of the surfaces and cross sections of EWP sheets with 35, 40 and 45 % of GLY and 0.48 water activity. The VP-SEM micrographs of the surfaces of the aforementioned EWP sheets are displayed in Figures 4.6a-c. White dots can be observed on the surface of the EWP sheets. These can be attributed to highly concentrated protein regions resulting from the non-homogeneous mix between the proteins of some parts of the protein network and the GLY. This claim seems to be supported by the reduction in the number of dots with the increased GLY content as the more GLY is available the more GLY can interact with the proteins and therefore, the less the amount of highly concentrated protein regions. The few white dots presented in Figures 4.6a-c indicate the low amount of highly concentrated protein regions and therefore, the homogeneous mix between proteins and GLY. The surface of the EWP sheets also exhibited protuberances. These were most probably caused when the surfaces of the sheets were wiped with a tissue prior to their freezing. This wiping was done to remove the

droplets formed on the surface of the sheets since these could have masked the sheet surface. The protuberances were less visible as the GLY content increased which shows that an increase in GLY content reduces damage caused by physical abrasion or others.

The VP-SEM micrographs of the cross sections of the aforementioned EWP sheets are displayed in Figures 4.6d-f. The cross sections of the fractured EWP sheets containing 35 and 40 % GLY (Figures 4.6d and 4.6e) are smoother than that of the 45 % GLY EWP sheet (Figure 4.6f) which shows marked grooves. These grooves are identified as river marks in the literature and are attributed to a ductile fracture. These grooves were likely formed due to extensive local deformation of the protein network as reported by Ogale et al. (2000) for compressed SPI-GLY films. Fractured surfaces with a silky and smooth texture like those presented in the cross sections of the EWP sheets containing 35 and 40 % GLY (Figures 4.6d and 4.6e) have been associated to a brittle failure where there is very little or no plastic deformation. Therefore, the higher the GLY content the lower the brittleness and the higher the flexibility of the EWP sheets. These results agree with those of the mechanical characterization of the EWP sheets where a significant reduction in E and a significant increase in E_b were observed with the increase in GLY content in EWP sheets with 0.48 water activity. The marks presented in the middle of the cross section of the EWP sheet containing 35 % GLY (Figure 4.6d) and to a much lesser extent in the cross section of the EWP sheet containing 40 % GLY (Figure 4.6e) could be attributed to the successive positions of the crack front as a function of time. The direction of the propagation of the crack in the EWP sheet containing 45 % GLY (Figure 4.6f) is shown by the direction of the river marks. Agglomerations of proteins are also observed in these micrographs (Figures 4.6d and 6e (small squares)). These decreased with the increase of GLY content and are not

observed in the cross section of the EWP sheet containing 45 % GLY (Figure 4.6f). This reduction of the protein agglomeration with the increase of GLY is also observed in the micrographs of the surfaces of the EWP sheets.

Summarizing, the increase in GLY content leads to EWP sheets that are more flexible, less rigid and stiff, more resistant to mechanical damage and results in a more homogenous mixture between the GLY and the proteins.

4.4. Conclusions

This study demonstrates that compressed egg white-based bioplastics with desired properties can be obtained by adjusting water activity and GLY content. Thus, lighter, more reddish and less yellowish sheets with a decreased thickness and second-order transition temperature, improved mechanical properties (increased flexibility and decreased rigidity and stiffness) and constant moisture content can be obtained by increasing water activity. Increasing the GLY content results in the same type of changes, but to a lesser extent and increased moisture content, while increasing both, water activity and GLY content, leads to a more pronounced effect in some of these properties.

Acknowledgements

APPENDIX

a_w	GLY content (%)	Moisture Content (%)	Thickness (mm)	Second second-order transition temperature (°C)
	35	$13.8 \pm 0.4^{Ba*}$	1.13 ± 0.02^{Aa}	175.8 ± 0.4^{Aa}
0.34	40	14.4 ± 0.3^{ABa}	0.95 ± 0.03^{Ba}	174.1 ± 2.6^{Aa}
	45	14.9 ± 0.4^{Aa}	0.90 ± 0.03^{Ca}	169.2 ± 3.9^{Aa}
	35	13.9 ± 0.4^{Ba}	0.76 ± 0.01^{Ab}	176.8 ± 1.9^{Aa}
0.48	40	14.9 ± 0.3^{Aa}	0.68 ± 0.01^{Bb}	159.6 ± 3.5^{Bb}
	45	15.6 ± 0.4^{Aa}	0.49 ± 0.02^{Cb}	152.8 ± 1.0^{Cb}

Table 4.1. Moisture content (%), thickness (mm), and second second-order thermal transition (°C) of EWP sheets with GLY contents of 35, 40 and 45 % and water activities (a_w) of 0.34 and 0.48 at 23.1 °C. The post-conditioning conditions were 55 % RH and 23.1 °C
*Different capital case letter indicate significant difference ($P \leq 0.05$) between EWP sheets with different glycerol contents and different small case letter indicate significant difference ($P \leq 0.05$) between EWP sheets pre-conditioned to different water activity.
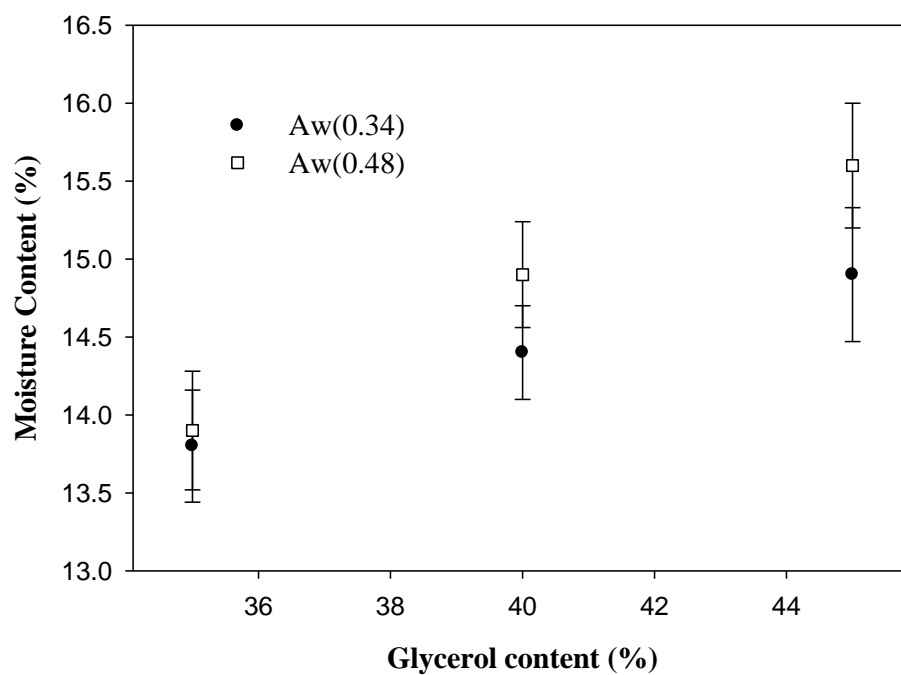
Figure 4.1. Moisture content (%) of EWP sheets with different GLY content (35, 40 and 45%) and water activities (0.34 and 0.48)
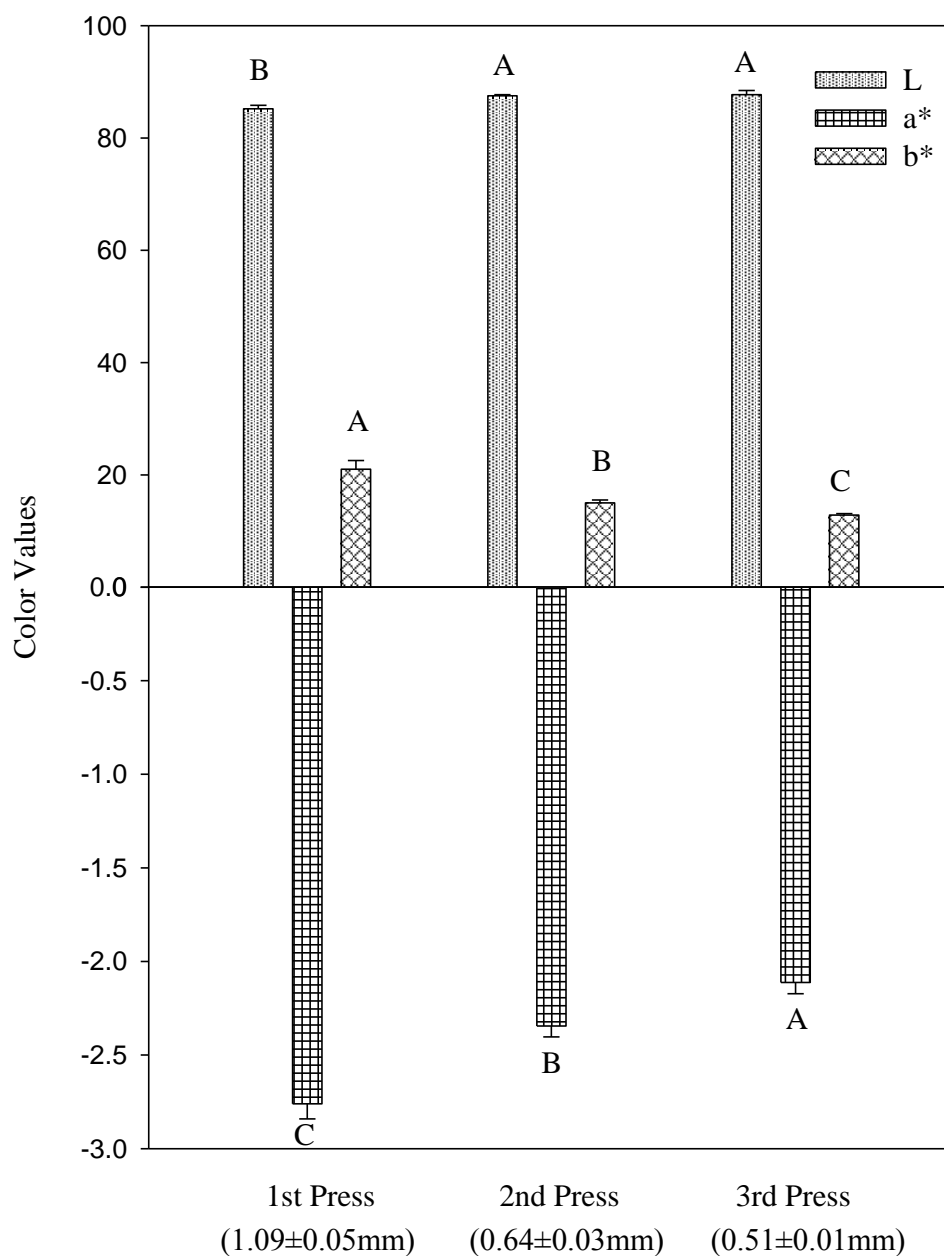
Figure 4.2. Lightness (L*) and color (a* and b*) of EWP sheets with 40 % GLY and 0.48 water activity after the 1st, 2nd and 3rd press. The sheets were post-conditioned at 55 % RH and 23.1 °C (Different capital case letter indicate significant difference ($P \leq 0.05$) between EWP sheets with different number of press)
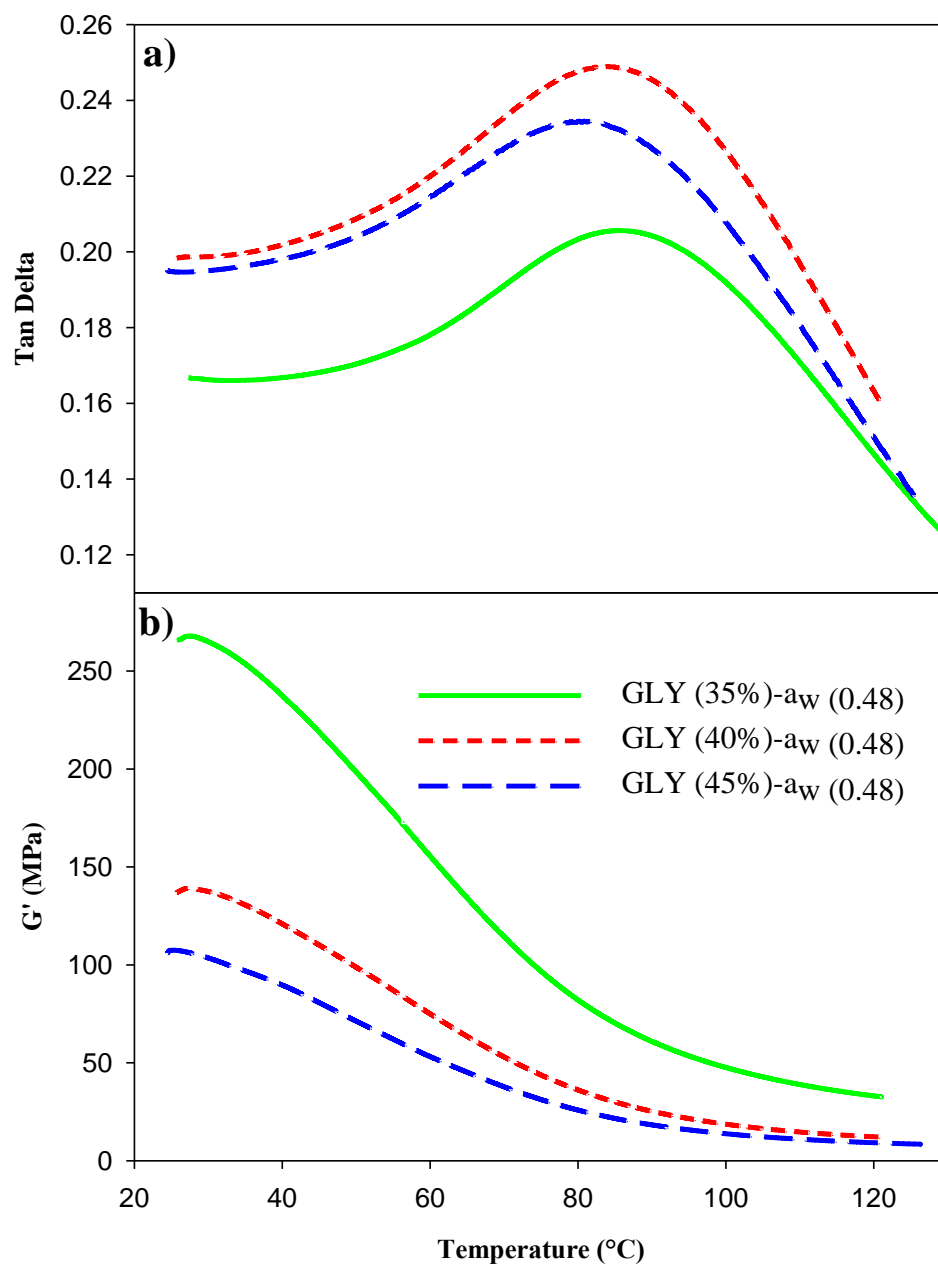
Figure 4.3. Tan Delta (a) and Storage modulus (G') (b) of EWP sheets with different GLY contents (35, 40 and 45 %) and pre-conditioned at 0.48 water activity at 23.1 °C. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of the thesis.
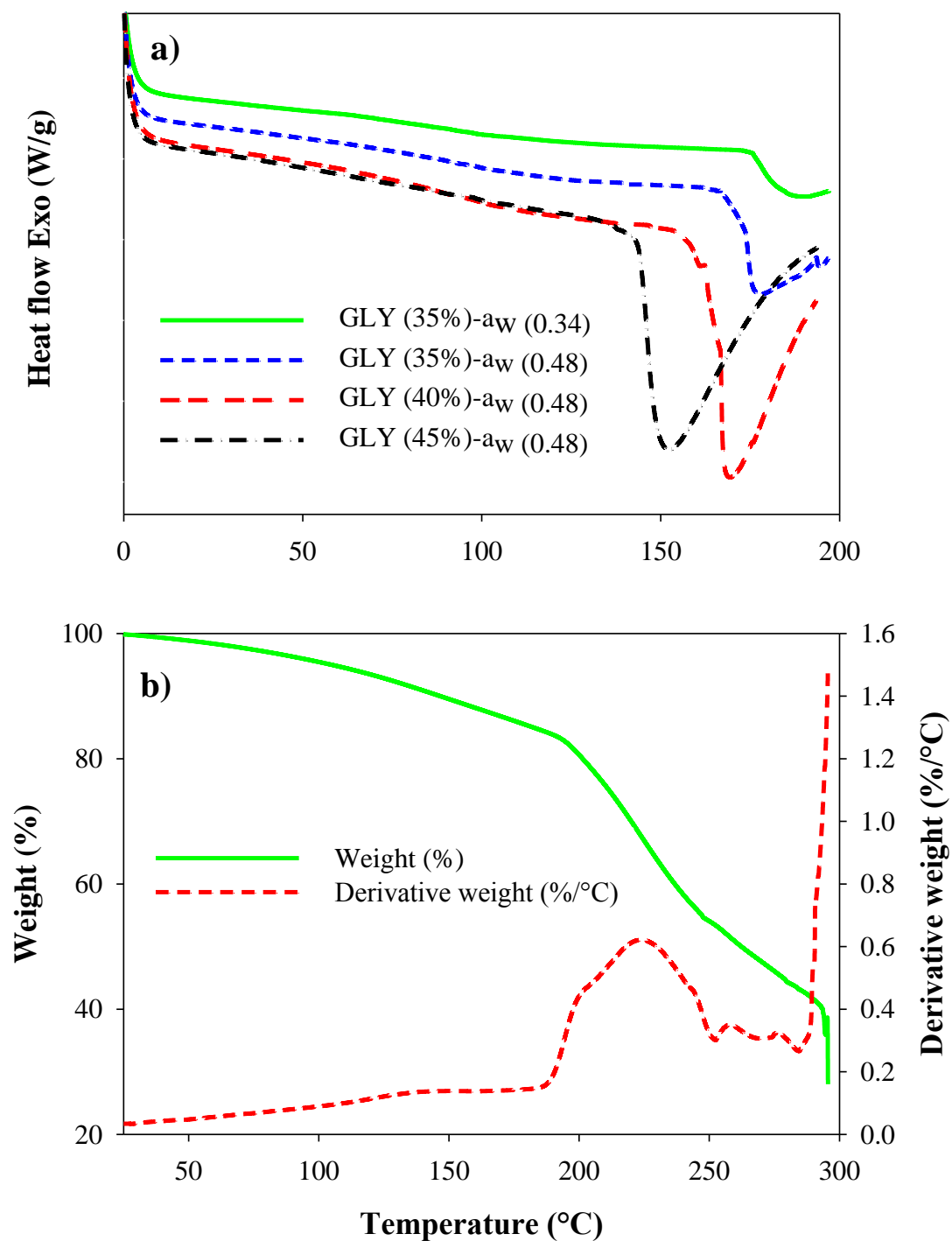
Figure 4.4. a) DSC thermograms of EWP sheets containing 35, 40 and 45 % GLY and 0.34 and 0.48 of water activity and b) TGA thermograms of EWP sheets containing 40 % GLY and 0.48 of water activity

a_w	GLY content	Color			Transmittance (%)
		L*	a*	b*	
0.34	35	84.9 ± 0.2^{Aa}	-2.74 ± 0.06^{Ba}	19.9 ± 0.4^{Aa}	N/a
	40	85.0 ± 0.4^{Aa}	-2.49 ± 0.05^{Aa}	19.5 ± 0.2^{Aa}	N/a
	45	84.6 ± 0.2^{Aa}	-2.40 ± 0.19^{Aa}	19.7 ± 0.5^{Aa}	N/a
0.48	35	87.0 ± 0.3^{Bb}	-2.27 ± 0.08^{Ab}	15.2 ± 0.5^{Ab}	89.4 ± 0.5^{A}
	40	86.9 ± 0.3^{Bb}	-2.08 ± 0.06^{Bb}	14.1 ± 0.9^{Bb}	89.2 ± 0.5^{A}
	45	88.7 ± 0.2^{Ab}	-1.88 ± 0.05^{Cb}	9.55 ± 0.3^{Cb}	89.0 ± 0.4^{A}

Table 4.2. Optical properties (lightness, color and transmittance) of EWP sheets with GLY contents of 35, 40 and 45 % and water activities of 0.34 and 0.48. EWP sheets were post-conditioned at 55 % RH and 23.1 °C.

*Different capital case letter indicate significant difference ($P \leq 0.05$) between EWP sheets with different glycerol contents and different small case letter indicate significant difference ($P \leq 0.05$) between EWP sheets pre-conditioned to different water activity. N/a denotes not measured.
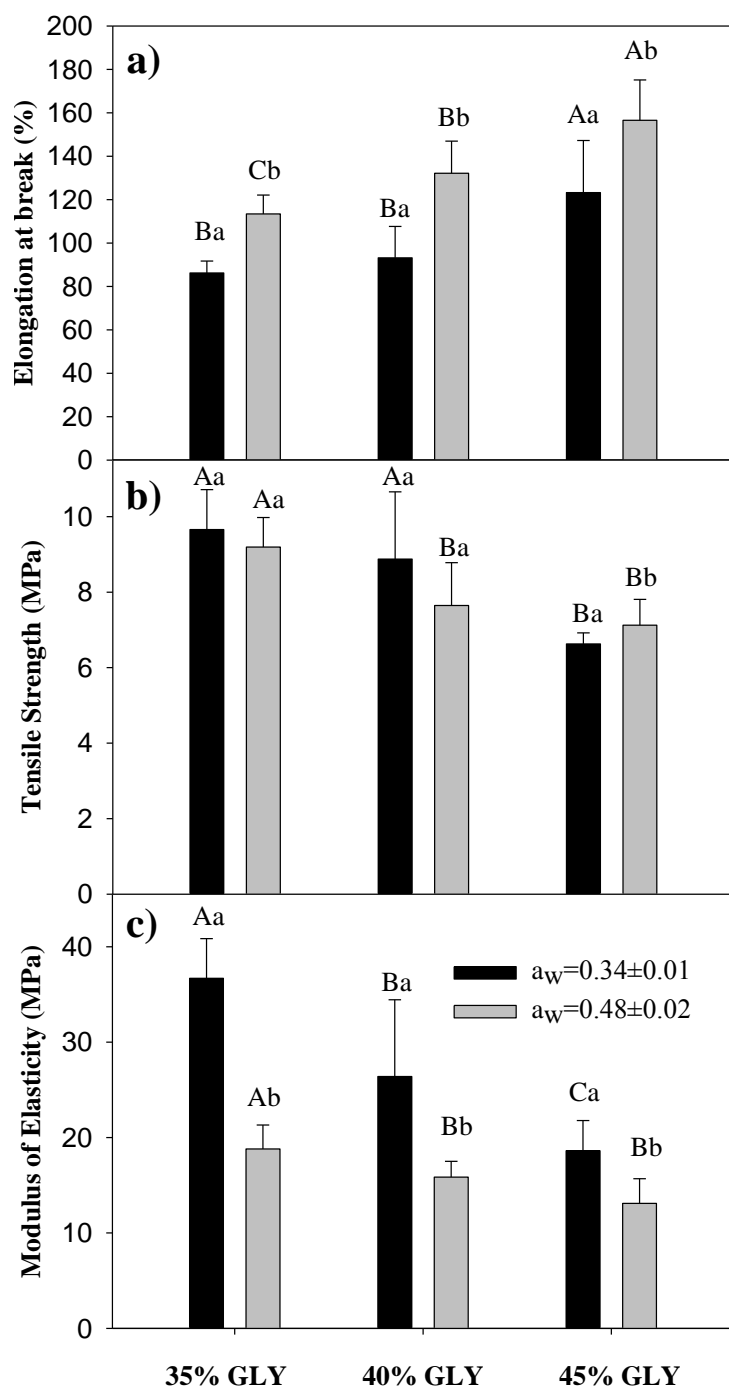
Figure 4.5. Elongation at break (%) (a), tensile strength (MPa) (b), and modulus of elasticity (MPa) (c) of EWP sheets with different GLY contents (35, 40 and 45 %) and water activities (0.34 ± 0.01 and 0.48 ± 0.02) (The sheets were post-conditioned at 55% RH and 23.1±0.1°C; different capital case letter indicate significant difference (P ≤ 0.05) between EWP sheets with different glycerol contents and different small case letter indicate significant difference (P ≤ 0.05) between EWP sheets pre-conditioned to different water activity)
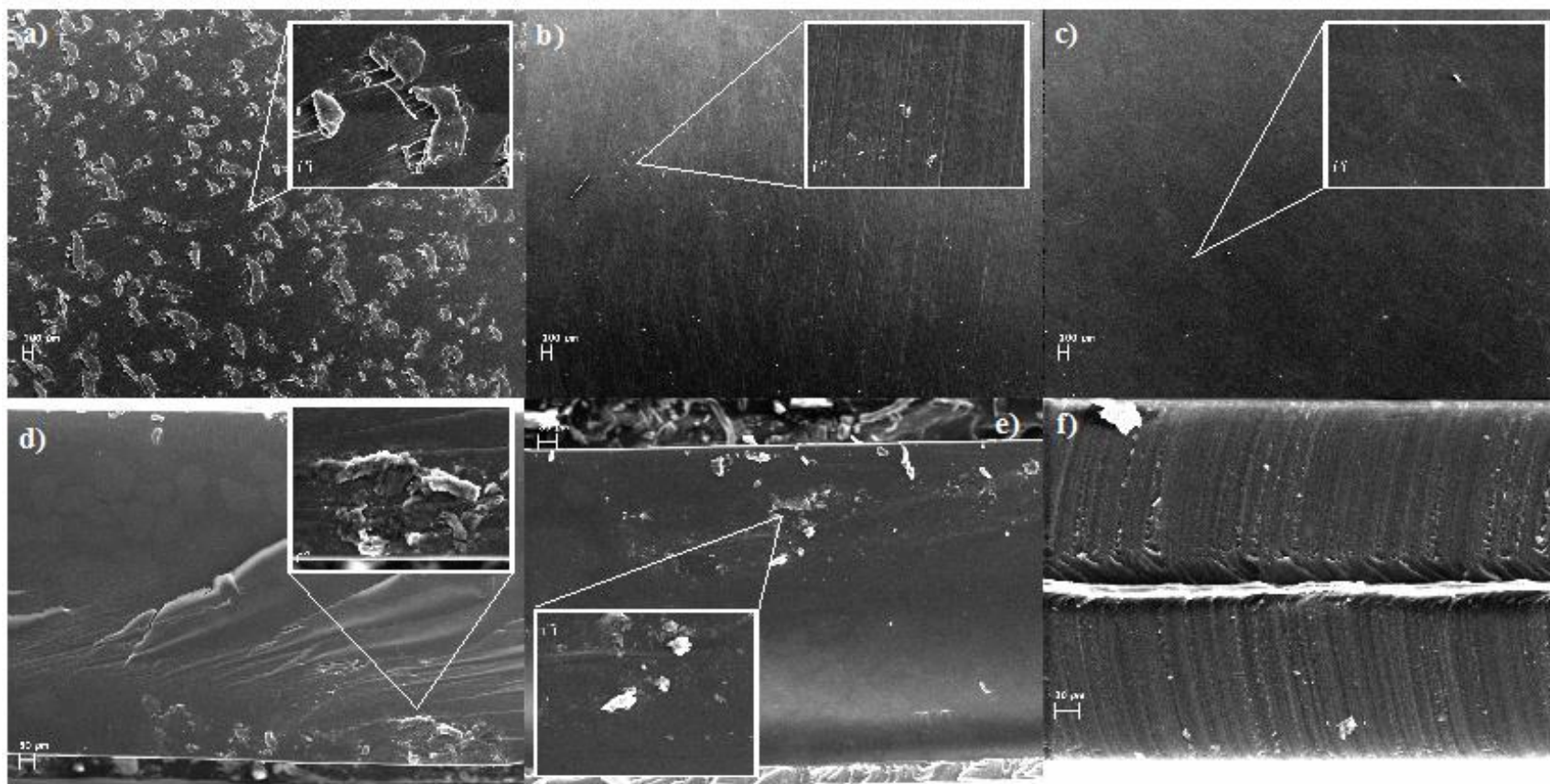
Figure 4.6. VP-SEM pictures of the surface of a) 35% GLY, b) 40 % GLY and c) 45 % and cross section area of d) 35 % GLY, e) 40% GLY and f) 45 % GLY EWP-sheets pre-conditioned at water activity of 0.48

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CHAPTER 5

CONCLUSIONS AND CONSIDERATIONS FOR FUTURE RESEARCH

5.1 Conclusions

The results of Chapter 3 show that the combination of GLY and water at specific levels can optimize the thermoplasticization of EWP. The compounding of EWP with GLY and water increased the capability of the EWP network to absorb water in sufficient amounts to allow the lowering of the second second-order transition temperature of the EWP network below the marked thermal degradation of EWP network. GLY was necessary for the thermoplasticization to occur but its increase from 35 to 45 % did not have a significant effect on the capability of the EWP network to absorb water and consequently, on the second second-order transition temperature. However, the increase of GLY in the EWP network improved the mixing between protein and plasticizer and reduced the EWP rate of decomposition. The increase in water activity (from 0.34 to 0.64) increased the capability of the EWP network to absorb water and consequently, lowered the second second-order transition temperature. A EWP network with a GLY content of 35 % and a water activity of 0.48 can be thermoplasticized at 150 °C without excessive degradation (less than 15 %). A maximum temperature of 170 °C and a maximum moisture content of about 12 % can be established for the adequate thermoplasticization of EWP. This study provides data useful for the appropriate compounding as a precursor step for the processing of EWP using industrial processes like compression and extrusion in order to develop films and sheets made from egg whites.

The results of Chapter 4 demonstrate that compressed egg white-based bioplastics with desired properties can be obtained by adjusting water activity and GLY content. Thus, lighter, more reddish and less yellowish sheets with a decreased thickness and transition temperature, improved mechanical properties (increased flexibility and decreased rigidity and stiffness) and constant moisture content can be obtained by increasing water activity. Increasing the GLY

content results in the same type of changes, but to a lesser extent and increased moisture content, while increasing both, water activity and GLY content, leads to a more pronounced effect in some of these properties.

5.2 Considerations for Future Research

Many new research interests and questions arose during this work. Some future areas of interest for packaging applications to be further investigated are:

1. The effect of different relative humidity on the mechanical, physical and barrier properties of compressed egg white-based bio-plastics. Proteins are hydrophilic in nature, thus it is important to investigate the effect of moisture on the properties of compressed based bioplastics made from egg white protein.

2. The changes in the mechanical, physical and barrier properties of the compressed egg white-based bioplastics caused by the sorption and desorption of different permeants such as ethanol, acetic acid, d-limonene, ethyl acetate, and acetaldehyde. This information will be useful for the understanding the behavior of egg white -based bioplastics when used for packaging food products.

3. The thermoforming and sealing capabilities of egg white-based bioplastics. This will enable us to make packaging containers from egg whites and to conduct food shelf life studies.

4. The extrusion of egg white-based bioplastics. The results of this thesis provide data useful for the optimal compounding of these proteins as a precursor step for the successful extrusion of egg white proteins. This will be a crucial step towards the use of egg white proteins in the packaging industry.

5. The use of other plasticizers such as sorbitol, oleic acid or PEG with different molecular weights should be investigated. Different plasticizers may impart different properties to the egg white-based bioplastics. These studies might provide useful information for the development of egg white-based bioplastics with desired properties.

6. The biodegradation of EWP-based bioplastics under composting conditions.