



VICIA VILLOSA PROTEIN ISOLATE: A NEW SOURCE OF PROTEIN TO MAKE A BIODEGRADABLE FILM

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ABSTRACT

Edible films from *Vicia villosa* protein isolate (VVPI) containing different contents of glycerol and sorbitol (30, 40, 50 and 60% w/w of protein) were developed. The aim of this study was to investigate the influence of type and concentration of plasticizers on the properties of edible films obtained from VVPI. Type and concentration of plasticizer significantly ($p < 0.05$) affected the mechanical, barrier, thermal and surface properties as well as opacity of the films. As plasticizer concentration increased, tensile strength decreased concomitant with increase in elongation at break and water vapor permeability. The similar trend behavior was observed for the film solubility, which increased with increasing plasticizer concentration. Sorbitol plasticized films, showed higher film solubility compared to glycerol plasticized films. Sorbitol plasticized films provided the most tensile strength values; however, its effect on water vapor permeability was low. In contrast, glycerol plasticized films exhibited the least tensile strength values, resulting in increasing water vapor permeability. Opacity of glycerol plasticized films was lower than that of sorbitol plasticized films, and decreased with increasing plasticizer content ($p < 0.05$). Also, a significant decrease ($p < 0.05$) was observed in thermal features and surface hydrophobicity values with increasing in plasticizer contents. It was observed that the films plasticized with sorbitol had lower moisture content than those with glycerol.

Keywords: biodegradable film; glycerol; sorbitol; *Vicia villosa* protein isolate

INTRODUCTION

The Current global consumption of plastics is more than 300 million tonnes, with an annual grow of approximately 5% (**Bioplastics market data, 2015**). Packaging is the biggest bazar for plastics, consuming more than 12 million tons per year. Although synthetic petrochemical-based polymers have been extensively applied in many packaging materials, they have become one of the main sources of waste after being utilized because of their weak biodegradability (**Rhim and Ng, 2007**). Plastic materials are not inert and where direct contact between the packed commodity and the plastic container occurs, there can be transfer of sufficiently mobile or soluble substances into the product as a result of a concentration gradient. These substances may be polymer additives and/or other adventitious impurities, such as monomers, catalyst remnants, polymer breakdown products and residual polymerization solvents. This transfer may introduce the risk of toxic hazard and/or formation of off-flavours (**Mannheim and Passy, 1990**). With the increasing demand of consumers for high quality foods and concerns on limited natural resources and the environment, the use of renewable resources to produce edible or biodegradable packaging materials that can maintain product quality and reduce

waste disposal problems are being explored (**Rhim and Ng, 2007**).

In order to prolong the postharvest shelf-life of fresh fruits and vegetables, edible films and coatings are able to supply a replacement for modified atmospheric packaging, because they could also lead to a rise in the shelf-life as in modified atmosphere storage during which the composition of the inside gas is adjusted (**Park, 1999**). Edible films and coatings could prevent moisture transfer between components of food package that have different water activity. Furthermore, biopolymer films are very good carriers for antioxidants, antimicrobial substances, pigments and other functional substances (**Haugaard et al., 2001; Petersen et al., 1999; Rhim and Ng, 2007**). Edible films and coatings after getting into nature decompose to water, carbon dioxide and inorganic compounds without any toxic residues in short time (**Haugaard et al., 2001; Petersen et al., 1999**).

In general, films comprised of one ingredient have either good barrier or good mechanical properties, yet not both. In fact, the valuable properties of various substances are gathered to form composite films: polysaccharides and proteins develop polymer interactions and create a network which is liable for the mechanical properties, however, they

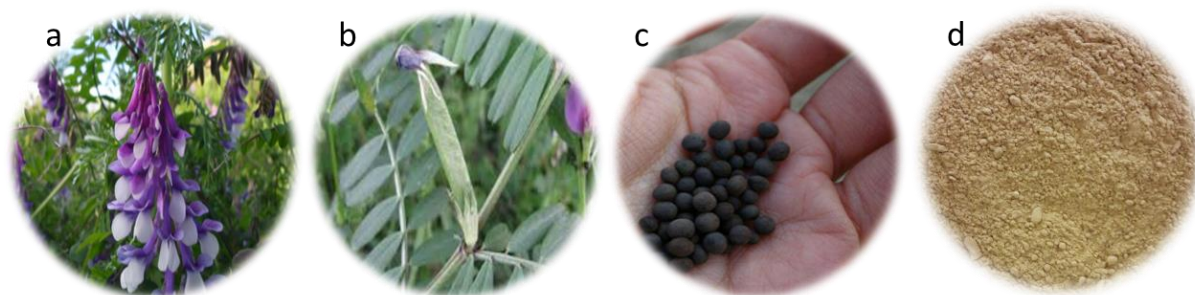


Figure 1 A pictorial view of (a) *Vicia villosa* plant, (b) cluster, (c) seeds, and (d) protein isolate.

are not appropriate water-vapor barriers due to their hydrophilic nature; in contrast, lipids cause the water-vapor barrier property of films owing to their hydrophobic nature; nevertheless, only the films made from lipids are almost brittle (Guilbert, 1986; Kester and Fennema, 1986).

Protein films have been recently considered as degradable and renewable. Proteins are traditionally employed in adhesives and as edible films/coatings. However, they possess a remarkable potential as the packaging films which degrade slowly. Proteins are interesting for polymer researchers since they have a variety of chemical functionalities, and are the molecules with numerous properties in nature (Petersen et al., 1999). Edible films resulting from various vegetable protein sources have been produced. These include corn zein, soy protein, wheat gluten, peanut protein, lentil protein, pea protein, faba bean protein and mung bean protein (Bamdad et al., 2006; Bourtoom, 2008; Choi and Han, 2001; Gennadios et al., 1993a; Liu et al., 2004; Otoni et al., 2016; Saremnezhad et al., 2010; Soliman et al., 2007).

Vicia villosa which is locally called Mashak-Gol-Khooshei in Iran; is one of the famous genera of Legumes. It has a large amount of energy and protein (20% ca.). *V. villosa* has up to 1 m long stems. It has covered with fine long (1 – 2 mm) hairs. Its leaflets are 5 – 10 pairs, narrowly oblong to linear-lanceolate and 1 – 2.5 cm long. Flowers of *V. villosa* are 10 – 30 arranged on one side of long-peduncled racemes. Their color are reddish-purple to violet with 1.5 – 1.8 cm long. Its legume has 2 – 3 cm long (Figure 1) (Aarssen et al., 1986; Shafiei et al., 2006; Tavili et al., 2010; Roy et al., 1968). The distribution of *V. villosa* is widespread throughout most of the United States, southern Canada (Gleason and Cronquist, 1963) and Iran (Tavili et al., 2010). In Alaska, it has been recorded above 60 °N (Hulten, 1968). Since this seed is an inexpensive source of protein, it has a good potential for the production of protein-based films. Despite the extensive research done in the field of edible protein films, there is no report regarding the film production of *V. villosa* protein isolate.

Scientific hypothesis

In this study, edible films from *Vicia villosa* protein isolate (VVPI) were prepared. Since Information on the effects of plasticizers on edible films from VVPI is poorly available at present, the aim of this investigation was to make a comparative study of different types of glycerol and sorbitol and their concentrations (30 – 60%) incorporated into edible films from VVPI. The different concentration of glycerol and sorbitol have an effect on the mechanical, barrier,

thermal and surface properties as well as opacity of the films.

MATERIAL AND METHODOLOGY

Materials

Vicia villosa seeds were purchased from a local market in Shahr-e-Kord, Iran. All reagents were of high-purity grade and used as received. Glycerol (CAS-no. 56-81-5, order no. 8.18709.1000) was obtained from Merck Millipore KGaA, Darmstadt, Germany. Sorbitol (NEOSORB® 70/70, CAS-no. 50-70-4) from Roquette GmbH, Frankfurt, Germany was used.

Isolation of VVPI

V. villosa seeds were cleaned manually to remove all extra matters. *V. villosa* seed flour was defatted using hexane (1 : 5 w/v) on a stirrer (60 min) prior to being milled and sieved. The defatted flour was dried in the ventilator at 20 °C for overnight. VVPI was prepared from defatted *V. villosa* seed flour by alkali method developed by Shahraki et al. (2013). Defatted flour was mixed with water at a ratio of 1 : 10 (w/v). The pH of the defatted flour suspended in water was adjusted to pH 9.9 using 1.0 M NaOH, continuously stirred with a magnetic stirrer for 1 h and centrifuged at 8000 g for 15 min. The pH of soluble phases were adjusted to pH 4.5 using 1.0 M HCl due to which proteins precipitates. The suspensions were centrifuged at 8000 g for 15 min, after which the supernatant was poured away and the precipitates were weighed and determined for protein content by the Kjeldahl method. Neutralization of precipitates was achieved by adjusting pH to 7.0 using 1.0 M NaOH, dialyzed by distilled water overnight at 4 °C and then vacuum dried and passed through 80 mesh sieve. The chemical composition of VVPI was determined according to AOAC (Horowitz and Latimer, 1994) standard methods.

Film preparation

Isolated *V. villosa* protein consists of 905.0 g kg⁻¹ protein content, 2.08% ash content, 0.3% crude fibre, 6.86% moisture, 0.08% fat and 0.81% carbohydrates. The film forming solution was prepared by dissolving VVPI in distilled water. Glycerol and/or Sorbitol were added at different proportions as plasticizers (Table 1). pH value of the solution was adjusted to 9 with 1 N NaOH. Alkaline pH range was selected to prepare films as VVPI is insoluble at acidic pH conditions, so it is not possible to prepare films at acidic pH with casting method. The solution was heated in water bath at 90 °C for 30 minutes, strained through muslin

Table 1 Composition of VVPI film-forming solutions prior to film casting.

Film	VVPI (g)	Plasticizer (g)	Plasticizer (%)	Water (g)
10% VVPI, 30% Glycerol	10	3	30%	87
10% VVPI, 40% Glycerol	10	4	40%	86
10% VVPI, 50% Glycerol	10	5	50%	85
10% VVPI, 60% Glycerol	10	6	60%	84
10% VVPI, 30% Sorbitol	10	3	30%	87
10% VVPI, 40% Sorbitol	10	4	40%	86
10% VVPI, 50% Sorbitol	10	5	50%	85
10% VVPI, 60% Sorbitol	10	6	60%	84

cloth, degassed under vacuum for 30 minutes and cast on Teflon-coated baking trays. The films were dried at 60 °C and 50 ±2% relative humidity for 8 hrs. Finally, the obtained VVPI films were peeled from plates and conditioned at ambient temperature in desiccators containing saturated solutions of Ca (NO₃)₂, 6H₂O (50 ±2% relative humidity, RH) for at least 48 h prior to tests. The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles and holes. Film failure was designated as loss of film cohesion resulting in cracking.

Film thickness

For each film sample nine thickness measurements were taken with a manual digital micrometer (Schwyz®, Zhejiang, China). Determinations were performed in triplicate. Averaged values of thickness measurements were obtained and these values were used in all calculations.

Moisture content

The moisture content (MC) of the films was determined after drying in an oven at 105 °C for 24 h. Pieces of films (15 × 7.5 mm) were cut after adequate conditioning and placed on test tubes previously weighed before and after oven drying. MC values were determined as a fraction of the initial film weight lost during drying and reported on a wet basis, according to the ASTM D-644 (ASTM, 1994). Determinations were performed in quintuplicate.

Film solubility

Film solubility in water was determined according to the method by Gontard et al. (1994) with modification. A piece of film sized 2.0 cm in diameter was cut, dried in an oven (DeLeo A1 SED, Porto Alegre, Brazil) at 105 °C to constant weight to obtain the initial film dry weight. Films were individually placed into 50 mL of distilled water and the mixture was shaken at a speed of 100 rpm using a shaker (Vicking, Buenos Aires, Argentina) at 25 °C for 24 h. The amount of dry matter in final samples was determined by drying at 105 °C to constant weight. The weight of solubilized dry matter was calculated by subtracting the weight of insolubilized dry matter from the initial weight of dry matter and expressed as the percentage of total weight. Film solubility represented total soluble matter dissolved in water, mainly including water-soluble proteins, glycerol and sorbitol.

Water vapor permeability

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh et al., 1993) was used to determine the water vapor permeability of films. The test cups were filled

with 20 g of silica gel (desiccant) to produce a 0% RH below the films. A sample of VVPI film was placed in between the cup and the ring cover of each cup coated with silicone sealant (LITHELEN, Leybold System GmbH, Germany) and held with four screws around the cup's circumference. The air gap was at approximately 1.0 cm between the film surface and desiccant. The water vapor transmission rates of each film were measured at 55 ±5% RH and 25 ±2 °C. After taking initial weight of the test cup, it was placed into an environmental chamber with an air velocity rate of 106.7 m.min⁻¹ (Incubator, Model KBF 115). Weight gain measurements were taken by weighing the test cup to the nearest 0.0001 g with an electronic scale (Sartorius Corp.) every 3 h for 18 h. A plot of weight gained versus time was used to determine the water vapor transmission rates. The slope of the linear portion of this plot represented the steady state amount of water vapor diffusing through the film per unit time (g.h⁻¹). Water vapor transmission rates were expressed in gram units, per square meter, per day. Steady state over time (slope) yielded a regression coefficient of 0.99 or greater. Nine samples per treatment were tested. The water vapor permeability of film was calculated by multiplying the steady water vapor transmission rates by the film thickness and dividing that by the water vapor pressure difference across the films.

Opacity

Opacity of films was measured according to the method of Gontard et al. (1992). In brief, films were cut in rectangular strips of 0.4 cm × 7.3 cm and placed in the spectrophotometer cell. A spectrum of each film was recorded using a UV-Vis spectrophotometer (Shimadzu UV-160A). The area under the absorbance curve from 400 to 800 nm was determined by Adobe Photoshop CS and defined as the film opacity.

Surface hydrophobicity

The sessile drop method, based on the optical contact angle, was used to estimate the surface hydrophobicity of the films. The contact angle (θ) was determined with a face contact angle meter OCA 20 (from Dataphysics, Filderstadt, Germany), according to Kwok and Newman (1999): a 2 µL-droplet of ultrapure water was deposited on the film surface with a 500 µL precision syringe (Hamilton, Bonaduz, Switzerland), using a needle with a diameter of 0.75 mm. Ten replicated measurements of θ were obtained.

Tensile strength and Elongation at break

Tensile strength was performed with an Instron universal testing instrument (LLOYD Instrument, Model LR30K,

Hants, England) as per ASTM D882-91 Standard Method (ASTM, 1995). Fifteen samples, 2.54 cm × 10 cm, were cut from each film. Initial grip separation and cross head speed were set at 50 mm and 50 mm/min, respectively (Ghasemlou et al., 2011).

Differential scanning calorimetry (DSC)

DSC measurements were performed with a Shimadzu DSC-50 calorimeter (from Shimadzu Corporation, Kyoto, Japan), equipped with STARe 6.1 Thermal Analysis System software. The instrument was calibrated with an indium standard, characterized by a T_m of 156.6 °C and a ΔH_m of 28.71 J.g⁻¹ (TA Instruments, New Castle DE, USA). Each sample was heated at a rate of 10 °C.min⁻¹, from -150 °C (assured with liquid nitrogen) to 250 °C, under an inert atmosphere (100 mL.min⁻¹ of N₂). The glass transition temperature (T_g) was recorded as the inflexion point of the baseline, caused by the discontinuity in the specific heat of the sample (Ghanbarzadeh and Oromiehi, 2008). The temperature of melting (T_m), observed as an endothermic peak, and the associated enthalpy (ΔH_m) were determined (and expressed as J.g⁻¹ protein) as reported by Ryan et al. (2008). These experiments were performed at least in duplicate, using punctured aluminum DSC pans (Al crimp Pan C.201-52090) containing 10 mg of dry sample. The samples were weighed with an automatic electrobalance AE 200 (from Mettler, Columbus OH, USA), with a precision of ±0.01 mg. An empty pan was used as reference.

Statistic analysis

A completely randomized experimental design was used to characterize the films. Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple comparisons were performed using Duncan's Multiple Range Test (DMRT). The statistical analysis of the data was performed using SPSS statistical software version 18 (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Normally, protein films need to be incorporated by some plasticizers to induce sufficient flexibility and to avoid cracking of the films during the drying or handling processes. The general function of plasticizers is to decrease inter and intra-molecular interactions among polymer chains, resulting in a rise in the spacing of free volume and make the molecules movements easier °C Lieberman and Gilbert, 1973). Therefore, the polymeric network becomes smooth and flexible. It is so necessary that the correct type

and content of plasticizer would be chosen for the preparation of a polymer film as its effect can significantly change the functional properties of the obtained products. The commercial plasticizers, namely glycerol and sorbitol, are added ranging from 0.2 to 1 g per 1 g of the film-forming agent (Chae and Heo, 1997; Cho and Rhee, 2002; Jongjareonrak et al., 2006; Osés et al., 2009; Ryu et al., 2002; Tanaka et al., 2001).

The present study revealed that the edible films of coherent VVPI were prepared when these plasticizers were incorporated at the concentrations between 20% and 70% (w/w of protein). However, the films obtained from VVPI containing 20% (w/w) sorbitol were brittle and broke easily during the removal from the casting surface. In turn, the films casted from VVPI containing 70% (w/w) glycerol were found to be very adhesive and too sticky to handle. Finally, in the work, the properties of films formed from VVPI containing 30–60% (w/w) glycerol and sorbitol were examined.

Film thickness values at the protein concentration 10% VVPI at different content of plasticizers 30%, 40%, 50% and 60% (w/w) showed similar values in thickness from 0.060 ±0.003 to 0.069 ±0.004 mm (Table 2). The differences in thickness were not significant ($p > 0.05$). Thickness of VVPI film did not affect by the glycerol and sorbitol concentration as also observed by Rodriguez et al. (2006).

Moisture content

MC of the films plasticized with different contents of glycerol and sorbitol are presented in Table 2. The films plasticized with sorbitol had lower moisture than those with glycerol ($p < 0.05$) could be due to the fact that sorbitol had ability to bind less water than glycerol thereby, provided lower MC. Addition et al. (1993a) reported that the rich of hydrophilicity of glycerol molecules, which is favorable to the adsorption of water molecules, could also be contribute to the increase in moisture in the films. This results show similar study of Chick and Ustanol (1998) who reported that casein-based films plasticized with glycerol had higher MC than films plasticized with sorbitol when the same amounts of plasticizers were used.

Water Solubility

The water solubility is also a major characteristic when selecting a film for specific applications (Arvanitoyannis et al., 1998). Generally, when the water solubility of a film is high, it cannot protect food from moisture or from water loss. For some uses, including packaging wraps, the high

Table 2 Values (average ± standard deviation) of thickness, moisture content and water solubility of VVPI-based edible films, with various glycerol and sorbitol contents.

Plasticizer type	Content % (w/w)	Thickness (mm)	Moisture content (%)	Water solubility (%)
Glycerol	30	0.060 ±0.003 ^a	21.47 ±0.89 ^c	48.11 ±1.09 ^d
	40	0.062 ±0.003 ^a	27.05 ±1.62 ^b	59.14 ±0.76 ^c
	50	0.064 ±0.002 ^a	31.56 ±2.17 ^{ab}	68.09 ±0.91 ^{bc}
	60	0.066 ±0.003 ^a	36.66 ±1.51 ^a	70.65 ±1.30 ^b
Sorbitol	30	0.066 ±0.002 ^a	16.11 ±0.91 ^e	60.66 ±0.50 ^c
	40	0.066 ±0.004 ^a	16.84 ±0.77 ^e	55.87 ±0.43 ^{cd}
	50	0.068 ±0.002 ^a	17.28 ±1.12 ^{de}	71.08 ±0.19 ^b
	60	0.069 ±0.004 ^a	18.12 ±0.42 ^d	80.34 ±0.74 ^a

Note: the values with the same letter are not significantly different at the $p < 0.05$.

solubility is a biodegradability index which can be taken into account as an advantage for such films (Stuchell and Krochta, 1994).

From visual observations and irrespective of plasticizer type and content, the edible films from VVPI clearly did not lose integrity after a 24 h immersion in water. Irrespective of the type, an increase in plasticizer content leads to an increase in films solubility (Table 2). It could be hastily concluded that hydrophilic plasticizers enhanced films solubility in water.

The lowest films solubility of edible films from VVPI plasticized by 30% w/w of these plasticizers were noticed, while increasing the amount of plasticizer content showed higher films solubility. Increasing glycerol concentration significantly altered the water solubility of VVPI-based film from 48.11 to 70.65% while increasing sorbitol altered it from 60.66 to 80.34% ($p < 0.05$) (Table 2). The highest solubility was obtained when the highest concentration of sorbitol (60%, w/w) was added. Plasticizers are capable of decreasing crosslinks among biopolymer molecules, thus increasing the film solubility (Ghasemlou et al., 2011). These results were similar to those achieved by Wittaya (2013), who also reported that increasing plasticizer content (glycerol and sorbitol) in mung bean protein films increased the film solubility.

Water Vapor Permeability

As a food packaging, it is normally needed that films avoid or at least decrease the moisture transfer between the food product and the surrounding medium, and water vapor permeability should be enough low (Ma et al., 2008). Water vapor permeability is a proportional constant presumed to be independent of the pressure gradient of water vapor used across the films. However, hydrophilic materials, such as protein films, deviate from this ideal behavior due to the interactions of permeating water molecules with polar groups in the film's structure (Hagenmaier and Shaw, 1990). Deviation from the ideal behavior can also be induced by the effects of structure on materials (Myers et al., 1961). Water vapor permeability of edible films from VVPI with different type and concentration of plasticizer were examined (Figure 2). The water vapor permeability increased with increasing of plasticizer concentration. This

tendency can be described by the structural changes in the protein network. The incorporation of plasticizers modified the molecular organization of the protein network, with an increase in free volume. The network becomes less dense and as a consequence more permeable (Ashley, 1985). Permeability increased with plasticizer content could be related to hydrophilicity of plasticizer molecules. Introducing hydrophilic plasticizers, favorable to adsorption and desorption of water molecules, has been reported to improve the water vapor permeability of hydrocolloid-based films (Gontard et al., 1993; McHugh et al., 1994). Comparing of the successive values of the water vapor permeability for each plasticized films was shown in Figure 2.

Films plasticized with sorbitol had lower water vapor permeability than those with glycerol at each plasticizer concentration ($p < 0.05$), respectively due to the fact that sorbitol had ability to bind less water than glycerol and, thereby, provided a lower water vapor permeability (McHugh et al., 1994). Chick and Ustunol (Chick and Ustunol, 1998) reported that casein-based films plasticized with glycerol had higher water vapor permeability values than films plasticized with sorbitol when the same amounts of plasticizers were used. The high hydrophilicity of glycerol molecules, which is favorable to the adsorption of water molecules, could also be contribute to the increase in the films water vapor permeability (Gennadios et al., 1993b). The increase in water vapor permeability with increasing hydrophilicity plasticizer concentration was also common in edible films (Cuq et al., 1997; McHugh and Krochta, 1994). Sorbal et al. (2001) reported that hydrophilicity of the plasticizers will increase the water content of the films, consequently increasing the mobility of the molecules. In addition, increasing water content could also affect permeate solubility in the films.

Film opacity

Film opacity is an important attribute in terms of food packaging, because transparency of packaging allows consumers to see the product before buying (Gontard et al., 1992; Orliac et al., 2003).

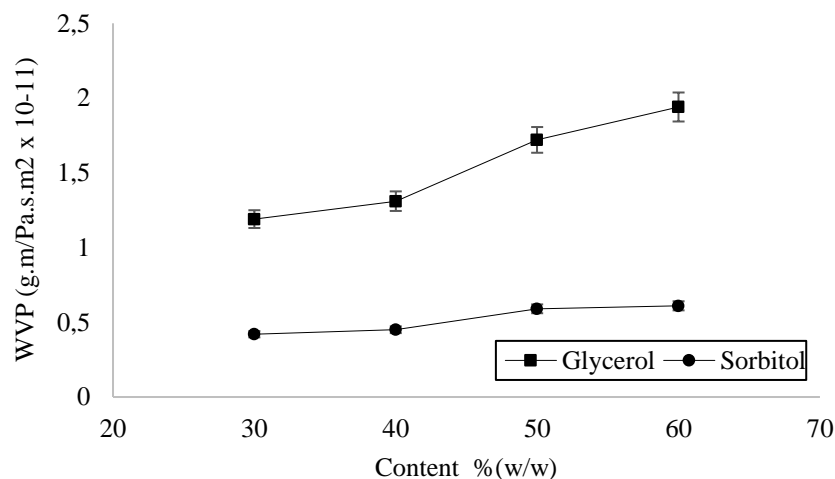


Figure 2 Water vapor permeability of 10 % (w/w) VVPI films in the presence of 30, 40, 50 and 60 % (w/w of VVPI) glycerol and sorbitol. Data represent the mean \pm one standard deviation ($n = 3$).

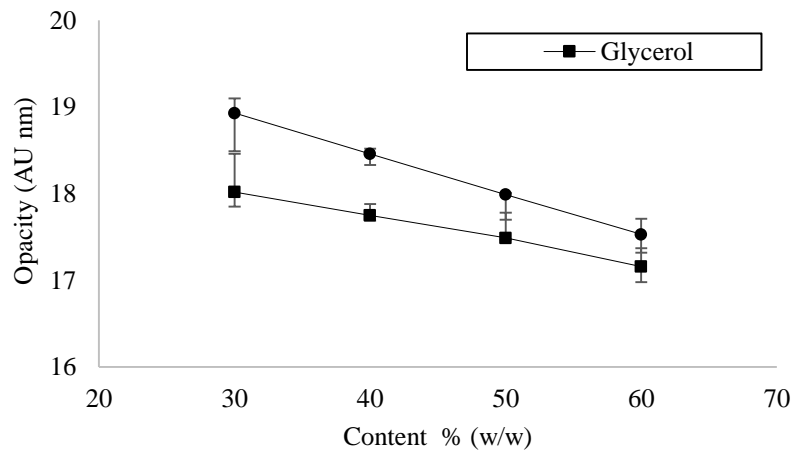


Figure 3 Opacity of 10 % (w/w) VVPI films in the presence of 30, 40, 50 and 60 % (w/w of VVPI) glycerol and sorbitol. Data represent the mean \pm one standard deviation ($n = 3$).

Film opacity was investigated as a function of plasticizer type and concentration and presented in Figure 3. An analysis of variance found the effect of plasticizer type and concentration was significant ($p < 0.05$), meaning that the transmission of light through the resulting films changed with plasticizer type and concentration. Overall, films prepared with glycerol were less opaque. Based on these findings, it was hypothesized that since the glycerol molecule was smaller than sorbitol, it was more homogeneously dispersed. In contrast, sorbitol was more heterogeneously dispersed causing light to scatter more.

This results show similar study of **Chang and Nickerson (2014)** who reported that canola protein isolate-based films plasticized with 50% sorbitol had higher opacity than films plasticized with glycerol when 1% (w/w of CPI) genipin was used.

These findings are important since film transparency or opacity are critical properties in various film applications, particularly if the film will be used as a surface food coating or for improving product appearance (**Gontard et al., 1992**). In many applications, an increased opacity is undesirable, although some applications need to provide protection against reactions of deterioration produced by the effect of light, offering some advantage to this type of film.

Surface hydrophobicity

Surface hydrophobicity of protein films was evaluated via measuring the contact angle of water (θ) upon the film surface by the sessile drop method. In general, films with higher (θ) values exhibit a higher surface hydrophobicity (**Tang and Jiang, 2007**); quantitative differentiation between “hydrophobic” and “hydrophilic” surfaces is indeed based on whether $\theta > 65$ or $\theta < 65$, respectively (**Vogler, 1998**).

From inspection of Figure 4, films containing 30% (w/w) sorbitol can be considered to have hydrophobic surfaces, since θ took values of $69.5 \pm 2.6^\circ$ and $65.8 \pm 2.1^\circ$ for the upper and lower surfaces, respectively. Conversely, VVPI-based film with the highest glycerol content (60%, w/w) could be considered to have the most hydrophilic surface. Furthermore, statistically significant differences ($p < 0.05$) were recorded between films containing different content of glycerol and sorbitol Figure 4.

It is also apparent in Figure 4 that θ (for the upper and lower surfaces) of VVPI films decreased proportionally to the increase in glycerol and sorbitol; once again, such a behavior was expected due to the hygroscopic nature of glycerol and sorbitol (**Sobral et al., 2001**).

This result is consistent with the claim by **Sobral et al. (2001)**, who reported that increasing concentrations of

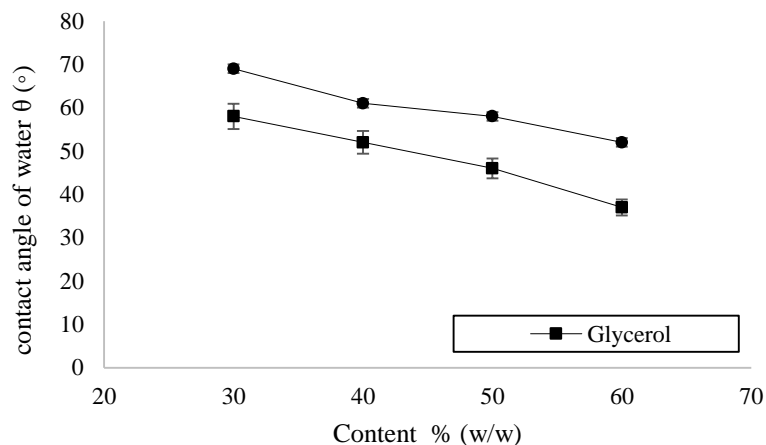


Figure 4 Water contact angle values (average \pm standard deviation, $n = 10$) for upper and lower surface of 10 (%w/w) VVPI-edible films, with various glycerol and sorbitol contents.

glycerol facilitate water absorption and transport within the films.

Tensile Strength and Elongation at Break

Edible films may be subjected to various types of stress during use; the determination of the mechanical properties involves not only scientific but also technological and practical aspects (Cagri et al., 2004). Tensile strength is the maximum tensile stress sustained by the sample during the tension test. If maximum tensile stress occurs at either the yield point or the breaking point, it is designated tensile strength at yield or at break respectively (ASTM, 1995). Elongation at break is an indication of a film's flexibility and stretch ability (extensibility). Preliminary work demonstrated that edible films from VVPI formed without plasticizer as relatively brittle and broke easily when peeled off. Hence desirable mechanical properties of edible films were improved by using two types of plasticizer (glycerol and sorbitol) at different concentrations (30, 40, 50 and 60%). The mechanical properties of films plasticized by glycerol and sorbitol at different concentration were assessed by measuring their tensile strength and elongation at break. The results are depicted in Figure 5. It was observed that an increase in the content of these plasticizers resulted in decrease in mechanical resistance (decrease in tensile strength) and trend to increase in extensibility

(increase in elongation at break). Glycerol and sorbitol are low molecular weight hydrophilic molecules that could easily fit into protein chains and establish hydrogen bonding with reactive groups of proteins. Bringing together plasticizers and proteins induced formation protein-plasticizer interactions to the detriment of protein-protein interactions. As a consequence, the density of intermolecular interaction in material decreased and the free volume between polymer chains increased (Cuq et al., 1997). The changes in mechanical properties as affected by hydrophilic plasticizers were observed for various hydrocolloid-based films (Gontard et al., 1993; Park and Chinnan, 1990). The mechanical properties of glycerol and sorbitol plasticized films at an equal concentration were compared (Figure 5).

The sorbitol plasticized films had significantly ($p < 0.05$) higher tensile strength and lower elongation at break than glycerol plasticized films at all concentrations. This could be attributed to the ring molecular conformation of sorbitol molecules, which may sterically hinder insertion between the protein chains resulted in less effective in disrupting the protein-protein interruptions. McHugh and Krochta (McHugh and Krochta, 1994) studied whey protein isolated/sorbitol (1 : 1) and whey protein isolated/glycerol (2 : 3) films and presented similar tensile strength values. They concluded that a higher amount of sorbitol than glycerol was needed to obtain similar tensile strength

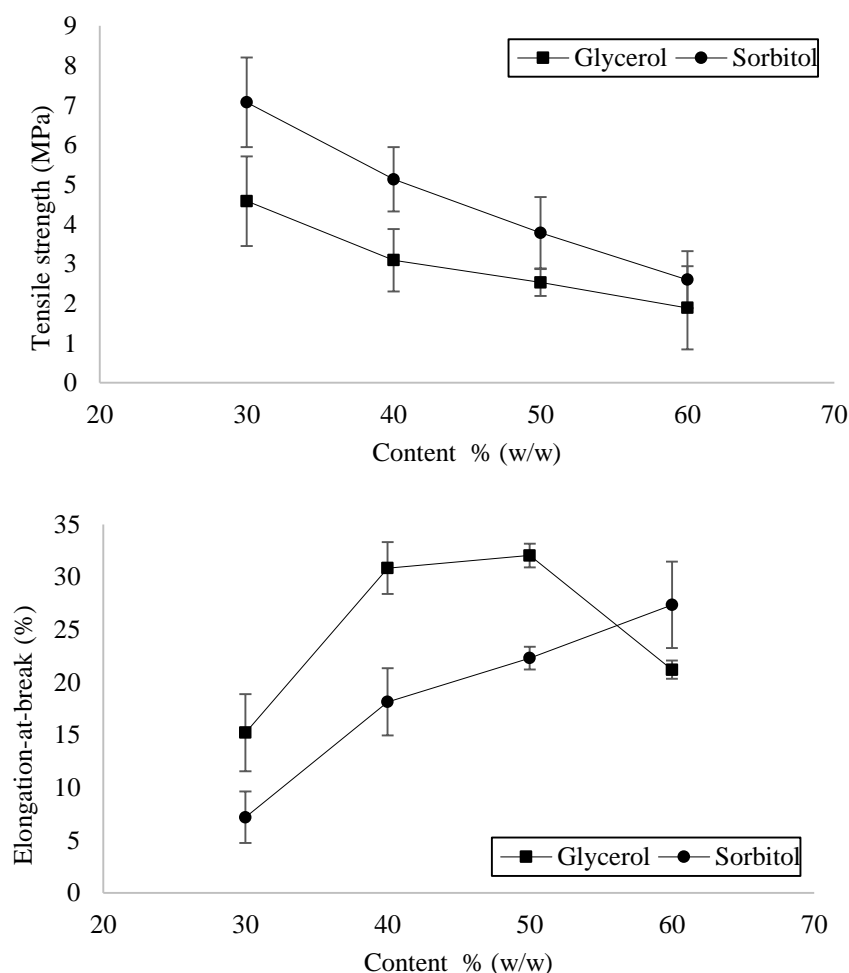


Figure 5 Tensile strength and Elongation at break of 10 % (w/w) VVPI films in the presence of 30, 40, 50 and 60 % (w/w of VVPI) glycerol and sorbitol. Data represent the mean \pm one standard deviation ($n = 3$).

Table 3 DSC measurement results of VVPI based Films with various glycerol and sorbitol contents, in terms of glass transition temperature (T_g), melting temperature (T_m), enthalpy of melting (ΔH_m).

Plasticizer type	Content % (w/w)	ΔH_m (J.g ⁻¹)	T_m (°C)	T_g (°C)
Glycerol	30	193.86 ± 2.13 ^b	172.85 ± 1.0 ^b	41.12 ± 1.56 ^c
	40	183.93 ± 1.45 ^{bc}	168.18 ± 1.28 ^b	36.43 ± 1.64 ^{cd}
	50	180.00 ± 1.12 ^c	152.91 ± 1.38 ^c	30.11 ± 0.97 ^d
	60	179.94 ± 2.47 ^c	149.30 ± 1.59 ^d	26.69 ± 1.85 ^e
Sorbitol	30	209.91 ± 2.42 ^a	184.55 ± 1.37 ^a	53.19 ± 1.54 ^a
	40	186.98 ± 1.55 ^{bc}	161.72 ± 1.0 ^c	52.61 ± 0.95 ^a
	50	181.00 ± 1.43 ^c	156.82 ± 2.9 ^{cd}	50.49 ± 1.09 ^{ab}
	60	180.94 ± 2.42 ^c	155.11 ± 1.61 ^{cd}	49.14 ± 1.12 ^b

Note: the values with the same letter are not significantly different at the $p < 0.05$.

properties. The glycerol plasticized films were more stretchable than the sorbitol plasticized films (Figure 4), suggesting that glycerol could be a more effective plasticizer in edible films than sorbitol. The effectiveness of glycerol in the edible films from VVPI are most likely due to its small size and configuration which allows it to be more readily inserted between the polymer chains, and consequently exert more influence on the mechanical properties than the larger molecule. **Donhowe and Fennema (1993)** found that plasticizer with low molecular weights such as glycerol was more effective than those with high molecular weights in methylcellulose-based films. Similarly, **McHugh and Krochta (1994)** suggested that smaller size plasticizer was more effective than larger size plasticizer in whey protein films. **Gennadios et al. (1993b)** reported that, the polar group (-OH) along plasticizer chains are believed to develop polymer-plasticizer hydrogen bonds replacing the polymer-polymer interaction in the biopolymer films. Molecular size, configuration and total number of functional hydroxide groups of the plasticizer as well as its compatibility with the polymer could affect the interactions between the plasticizer and the polymer (**Yang and Paulson, 2000**).

Thermal properties

DSC studies of VVPI films containing different concentrations of glycerol were performed to better understand the structure and interaction between polymers and plasticizer. The glass transition temperature (T_g) is the temperature at which the material under-goes a structural transition from the glassy state to a rubbery state (**Yang and Paulson, 2000**). Below T_g , films are rigid and brittle, whereas above it films become flexible and pliable.

The properties of VVPI films, at various levels of glycerol and sorbitol, were also analyzed in terms of thermal performance via DSC.

DSC thermograms showed two thermal transitions for VVPI films, irrespective of their content of glycerol and sorbitol; a glass transition for the amorphous fraction, and a melting transition for the crystalline one. The glass transition temperature (T_g), the melting temperature (T_m) and the melting enthalpy (ΔH_m) values are summarized in Table 3.

VVPI protein films containing sorbitol exhibited T_g , T_m and ΔH_m values significantly higher ($p < 0.05$) than those obtained for VVPI protein films containing glycerol, at a given content of glycerol and sorbitol (Table 3), thus suggesting stronger films.

From inspection of Table 3, it is possible to conclude that T_g and T_m decreased as glycerol and sorbitol content increased from 30 to 60% (w/w) ($p < 0.05$). This trend is a consequence of the plasticizing effect of glycerol and sorbitol molecules which typically increase the free volume of the polymer network and the segmental mobility of the polymer chains, thus decreasing both T_g and T_m (**Sobral et al., 2002, 2001**).

Table 3 showed that ΔH_m also decreased when the glycerol and sorbitol content increased, this decrease was statistically significant ($p < 0.05$). Such a decrease in thermal stability was affected by the presence of glycerol and sorbitol, which reduced the interaction between proteins, and thus stabilized the network structure (**Barreto et al., 2003**); in other words, higher glycerol and sorbitol content required a lower enthalpy to disrupt inter-chain interactions.

In addition, results suggest that glycerol and sorbitol were compatible with VVPI, and confirmed the effectiveness of plasticization since only one T_g followed by an endothermic peak (T_m) was observed (**Sobral et al., 2002, 2001**). If a polymer and the plasticizer, or two different polymers were immiscible, the mixture would in fact exhibit two T_g values, corresponding to the two pure phases (**Arvanitoyannis et al., 1997; De Carvalho and Grosso, 2004; Vanin et al., 2005**).

This result is similar to those obtained by **Ramos et al. (2013)**. They reported that the lower T_g , T_m and ΔH_m values of plasticized whey protein isolate (WPI) and whey protein concentrate (WPC) edible films could be attributed to the reduction of intermolecular forces and increase in the mobility of polymer chains.

CONCLUSION

The results of this study pointed out that as plasticizer concentration increased, tensile strength decreased concomitant with increase in elongation at break and water vapor permeability of VVPI films. Sorbitol plasticized films provided the films with highest mechanical resistance, but the poorest film flexibility. In contrast, glycerol plasticized films exhibited flexible structure; however, the mechanical resistance was low, while inversely affecting the water vapor permeability. Increasing the plasticizer concentration from 30 to 60% resulted in higher solubility (from 48.11 to 70.65 for glycerol and from 60.66 to 80.34 for sorbitol). Sorbitol plasticized films, showed higher film solubility, opacity, thermal features and surface hydrophobicity values compared to glycerol plasticized films. Addition it was found that the films plasticized with sorbitol had lower moisture content (16.11 – 18.12%) than those with glycerol (21.47 – 36.66%). Overall, the results of present study

indicated that adding of these plasticizers seems to be useful to fabricate VVPI films with acceptable characteristics. Therefore, the results revealed substantial potential of VVPI edible films to be incorporated in food packaging applications, especially for those that require less hydrophobic films.

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