Physicochemical and mechanical properties of edible films based on WPI and a novel hydrocolloid: Persian gum

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Abstract

In this study, the effect of Persian gum (PG) as a novel gum to be used in edible films was examined. Sorbitol-plasticized whey protein isolate (WPI) films with incorporation of PG in different ratios (100:0, 80:20, 60:40, 50:50, 40:60 and 20:80 WPI: PG) were prepared. The effects of PG concentration on the physicochemical properties; water vapour permeability (WVP), moisture sorption isotherms and mechanical properties; tensile strength (σ_{max}) and elongation at break (%EB) were observed. Incorporation of PG in the film matrix significantly increased WVP ($p<0.05$) when added in ratios 60:40, 50:50, 40:60 and further increased in the ratio of 20:80 (WPI: PG). The moisture uptake of the films was not greatly influenced by the increment of PG. Addition of PG in the lowest concentration of 80:20 (WPI: PG) highly decreased the tensile strength of the films; however further addition of PG did not cause any great decrement. A significantly higher elongation was observed especially when PG was present in ratios 40:60 and 20:80 (WPI: PG). Overall, incorporation of PG in sorbitol-plasticized WPI films affected positively the elongation characteristics. However, improvement is needed for WVP to retain more stable values. This study suggests further examination of the soluble and insoluble fractions of PG using different polymers and plasticizers. A study on the antimicrobial properties of PG in edible films could also be examined since PG is traditionally used as a remedy.

Edible films; Persian gum; Whey protein isolate; Sorbitol; Physical and mechanical properties

Introduction

Food packaging is essential for enhancing the quality, safety and shelf life of a foodstuff by protecting it from external factors that may cause spoilage. Plastic is the most common material used for packaging due to its low manufacturing cost and good physicochemical and mechanical properties it shows. Every year, it is estimated that the production of plastics is increasing by approximately 4%, reaching a rise from 1.5 Mt in 1950, to 335 Mt in 2016 (PEMRG, 2015; PEMRG, 2017). The major sector of plastics' application is packaging, which represented the 39.9% of the total plastics production in 2016 (PEMRG, 2017). The majority of the materials used for plastic packaging are made of petroleum which makes them non-biodegradable. As argued by Geyer, Jambeck and Law (2017), in 2015 approximately 6300 Mt of plastic waste was yielded, out of 79% (4977 Mt) was heaped in the landfills or the natural environment. The research estimates that if plastics production and waste management proceeds with the same pattern, by 2050 an estimated number of 12000 Mt of plastic waste will accumulate in the natural environment, causing a threat to fauna and greatly affecting greenhouse gas emissions hence the climate change (Geyer, Jambeck and Law, 2017).

Biopolymer-based packaging is one of the sectors which has attracted the interest of packaging industry due to its low energy consumption and low cost of manufacturing by utilizing food waste or by-products. In the last decades, a wide range of biopolymers consisted of different materials have been examined, aiming in the identification of ingredients and concentrations that will provide optimum performance in biopolymer-based packaging. In this manner, several novel materials are also used; Persian gum (PG)

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is a novel gum which has recently been characterized and its properties have not been widely examined (Behradmehr and Ahrari, 2014).

PG is exuded in the form of teardrops from the Iranian indigenous wild almond tree Amygdalus scoparia Spach (Dabestani *et al.*, 2018). PG was characterized by the use of gas chromatography and mass spectrometry and has shown to be consisted mainly of arabinose and galactose in a ratio of 2:1 with predominantly β-glycosidic linkages (Dabestani *et al.*, 2018). PG is partially soluble, consisting of a 70% w/w insoluble fraction (IFPG) and 30% w/w soluble fraction (SFPG). The soluble part can be dissolved in cold water, whereas the insoluble part can be partly dissolved in hot water (Dabestani *et al.*, 2018). Despite PG's 70% insolubility, Teimouri, Abbasi and Sheikh (2016) the effect of various levels of gamma irradiation (0, 4, 8, 16, 30 kGy revealed that treatment of PG with gamma irradiation of up to 16 kGy greatly increased the solubility of IFPG up to 88%, possibly due to the degradation of glycosidic bonds or the reduction of side branches.

PG is gaining interest in the past few years due to its abundance, low price and sustainability which may replace gums such as tragacanth and Arabic which are higher in cost and unsustainable, respectively (Abbasi, 2017). Traditionally PG is used in countries of central Asia as a herbal remedy for different purposes (Abbasi, 2017). Concerning PG's film-forming ability, a study revealed that composite of SFPG (3%) and glycerol (4%) was able to produce a film, but on the contrary, PG as whole and IFPG showed no film forming ability even in the presence of various metal ions and plasticizers (Dabestani *et al.*, 2018).

The aim of this study was the development of composite edible films made of Persian gum (PG), whey protein isolate (WPI) and sorbitol as a plasticizer, and the examination of their physicochemical properties; water vapour permeability and moisture uptake, and mechanical properties; tensile strength and elongation at break.

Materials and methods

Film preparation

An amount of 5% (w/w) whey protein isolate (WPI) of >95% purity (ARLA Food Ingredients, Denmark) was dissolved in distilled water under continuous stirring. The protein solutions were placed in a stirring water bath at 90°C and 150rpm for 30 minutes. Heating was applied for inducing partial denaturation of whey protein by the formation of intermolecular disulphide bonds; this process establishes a cross-linked polymeric network and a flexible film that keeps its structural integrity even at high moisture environments (Zinoviadou, 2013). The solutions were then rapidly cooled down to room temperature by the use of an ice water bath in order for further denaturation to be inhibited. Sorbitol (Duchefa Biochemie B.V., The Netherlands) was added as a plasticizer under continuous stirring, in 3% (w/w) concentration, for improving the brittleness of the films; sorbitol was used instead of another plasticizer as studies have shown better compatibility with WPI (Mchught and Krochta, 1994; Bozdemir and Tutas, 2003; Ramos *et al.*, 2012). The solution was placed at 4 °C overnight for air globules to be removed.

Persian gum (PG) (Freer Corp., Iran) was dissolved in distilled water under stirring and heating in concentration of 4% (w/w). Heating was applied as studies have argued that the solubility of the insoluble fraction is increasing when heating is applied (Teimouri, Abbasi and Sheikh, 2016; Dabestani *et al.*, 2018) structural and functional characteristics (e.g., emulsifying properties. The solution was left to reach 25ºC and sonication was applied at 20 kHz frequency and 70% amplitude, using an ultrasonic processor VC 750 (Sonics, USA). Ultrasound was performed for the solubility of the insoluble fraction to be further increased. Sorbitol (Duchefa Biochemie B.V., The Netherlands) was added as a plasticizer under continuous stirring, in 3% (w/w) for improving the brittleness of the films. The solution was placed at 4 °C overnight for air globules to be removed.

Solutions in ratios of 100:0, 80:20, 60:40, 50:50, 40:60, 20:80 and 0:100 WPI: PG were prepared under stirring. Sodium azide was added in amount of 0.02% for moulds growth to be inhibited. Portions of 15g of the solutions were casted in Petri dishes (φ 8.5 cm), were allowed to dry in a WTB oven at 45°C (BINDER GmbH, Germany) for approximately 12 hours and were stored under ambient conditions for 24 hours (Ket-on, Pongmongkol and Tananuwong, 2016). The solution of 0:100 WPI: PG was not able to form a film due to high adhesiveness and insufficient air globules' removal which resulted from the high viscosity and air entrapping in the solution.

Water vapour permeability (WVP)

The WVP was measured based on the ASTM (E96-63T) method with a modification of the vapour pressure at film underside according to Mchugh, Avena-bustillos and Krochta (1993). At least five film replicates

were equilibrated in an HPP 110 constant climate chamber (Memmert GmbH + Co. KG, Germany) at 25ºC and 53% RH for 48 hours. The films were sealed in cups containing distilled water and the steady–state water vapour flow was reached within one hour for all films (Zinoviadou, Koutsoumanis and Biliaderis, 2009). Film permeability was determined according to Kristo, Biliaderis and Zampraka (2007). The thickness was measured in five different spots using a manual micrometre and the average value was obtained. Slopes were calculated by linear regression and correlation coefficients for all reported data were > 0.95.

Moisture sorption isotherms

Moisture sorption isotherms were determined according to Biliaderis, Lazaridou and Arvanitoyannis (1999). Film samples of approximately 150mg were placed in previously weighted aluminium dishes and were dried in a WTB oven (BINDER GmbH, Germany) at 45ºC for two hours, for a constant weight to be reached. The samples were stored for 21 days at 25°C in desiccators containing saturated salt solutions of potassium acetate, potassium carbonate, magnesium nitrate and sodium chloride of known relative humidity; 23%, 43%, 53% and 75% RH, respectively. This time was necessary for constant weight and practical equilibrium to be reached. After storage, the moisture content was calculated by drying the samples at 105°C for two hours using a WTB oven (BINDER GmbH, Germany). Three replicates of each RH were measured for moisture sorption isotherms.

Mechanical properties

Films were cut in dumbbell-form strips of 1 cm width and 2 cm length, and were equilibrated in desiccators of controlled RH environments (23%, 43%, 53% and 75%) at 25ºC for a minimum of ten days in order for films of different moisture contents to be obtained. The thickness of the films was measured at two different points using a manual micrometre and the average value was calculated. Samples were analysed at ambient temperature using a CT3 texture analyser (Brookfield, USA) and a TA-TGF probe, set in tensile mode and crosshead speed of 60 mm× min^{-1}. The tensile strength (σ_{max}) and percentage of elongation at break (% EB) were calculated from the load-deformation curves of tensile testing (Lazaridou, Biliaderis and Kontogiorgos, 2003; Prommakool *et al.*, 2011). At least ten replicates of each RH were tested. After storage, the moisture content of the samples was measured by drying at 105 ºC for 2 hours using a WTB oven (BINDER GmbH, Germany).

Statistical analysis

All data were analysed by the general linear model (GLM) procedure of the SPSS software, (Version 25.0, IBM Corporation, USA). Comparisons were made using the Duncan's multiple range test to determine any significant differences among the samples at a 95% confidence interval.

Results And Discussion

Water vapour permeability (WVP)

A desired WVP prevents moisture transfer from the food to the surroundings, inhibiting thus any alteration in the quality of the product (Khezerlou *et al.*, 2018). The WVP of the WPI films containing no PG (9.56 ± 0.5 g-mm/h-m^2-kPa) were similar to the values reported by Anker et al. (1998) and Zinoviadou et al. (2013) who tested sorbitol-plasticized WPI films under similar concentrations and RH conditions.

As the data in (Plate V, Fig. 1) presents, WVP was increasing as the PG concentration was increasing. Compared to the values of the ratios 100:0 and 80:20, the WVP of the films was significantly increased ($p<0.05$) when PG was present in ratios 60:40, 50:50 and 40:60, whereas statistically significant difference ($p<0.05$) compared to the rest of the samples was observed at the ratio of 20:80.

Such results are expected in hydrophilic-rich films, as those of the current study, in which water vapour molecules are allowed to be dissolved and permeate through the film (Khezerlou *et al.*, 2018; Tonyali, Cikrikci and Halil, 2018). Increase in WVP may have been also assisted due to the increased air globules thus larger pores formation when PG was incorporated in higher amounts (Gounga, Xu and Wang, 2007).

Significant increase in WVP when PG concentration was increasing was also observed by Khezerlou et al. (2018) who examined the WVP of glycerol-plasticized sodium caseinate and PG composited films. In addition, Ramos *et al.* (2012) have argued that as the plasticizer concentration rises, the WVP also does. The amount of a compatible plasticizer in a matrix is crucial since the increment results in weaker interchain attractive forces (Zinoviadou, Koutsoumanis and Biliaderis, 2009). The effect of sorbitol

compared to glycerol on WVP was marked from Ramos *et al.* (2012) when tested the two plasticizers in WPI films; under equal to the present study's conditions the report found that sorbitol exhibited significantly lower WVP (0.9 g-mm/h-m^2-kPa) compared to the same amount of glycerol (6.4 g-mm/h-m^2-kPa). Sorbitol is of greater molecular weight and exhibits lower polarity than glycerol, therefore the lower polarity may be beneficial for the WVP to decrease (Mchught and Krochta, 1994).

Moisture sorption isotherms

Sorbitol-plasticized films of different WPI: PG ratios (100:0, 80:20, 60:40, 50:50, 40:60, and 20:80) were examined for water sorption isotherms under different RH (23%, 43%, 53% and 75%) environments. In all samples the moisture uptake was increasing as the RH was increasing (Fig. 2). Such behaviour is expected in films rich in hydrophilic compounds, as in the present case in which WPI was predominant (Zinoviadou, Koutsoumanis and Biliaderis, 2009b; Khezerlou *et al.*, 2018b). As observed in (Plate V, Fig. 2), despite the short increase in moisture uptake as the PG concentration was increased, PG indicated no great influence on the moisture uptake of the films.

The moisture sorption isotherms findings of 100:0 (WPI:PG) were analogous to those of Zinoviadou, Koutsoumanis and Biliaderis (2009b) when examined sorbitol-plasticized WPI films of similar concentrations, and to Coupland *et al.* (2000) who used glycerol as plasticizer in WPI films. According to Biliaderis, Lazaridou and Arvanitoyannis (1999), when plasticizer is absent in the film matrix moisture uptake values are displayed to be lower compared to those in which plasticizer is present. This is occurred due to the hydrophilicity of plasticizers from the hydroxyl groups which contain. A higher moisture affinity of glycerol compared to sorbitol was argued by Cho and Rhee (2002). This is explained as glycerol contains a higher amount of hydroxyl groups than sorbitol, creating to moisture molecules a more favourable environment for being adsorbed (Cho and Rhee, 2002).

Mechanical properties

Edible films are often subjected to external stresses and impacts during food packaging and handling thus their mechanical properties are of predominant importance. As depicted in (Plate VI, Fig. 3), addition of PG even in the lowest concentration of 80:20 (WPI: PG) highly decreased the tensile strength of the films when moisture content was above 8%. Increment of moisture content and further addition of PG caused a constant decrease on the σ_{max}, however such decrease was not significant ($p > 0.05$).

At a moisture level of 11.3%, the sample containing no PG exhibited a value of 15.1 MPa, similar to the values argued from Ozdemir and Floros, 2008; Osés *et al.*, 2009; Zinoviadou, 2013b when examined WPI films containing equivalent amounts of sorbitol. A study by Zinoviadou (2013) who applied additional lower RH environments (11%), observed an increase in stiffness when the samples exhibited moisture content below 8%. The reason behind this as explained in previous studies (Pittia and Sacchetti, 2008; Zinoviadou, Koutsoumanis and Biliaderis, 2015) is due to the fact that water exerts a strong plasticizing action on hydrophilic polymers in low-moisture systems, causing a large depression of their glass transition temperature. Based on the aforementioned, a lower value of tensile strength would be therefore expected if the samples were examined in lower moisture. Nevertheless, when moisture reaches moderate levels water acts as a plasticizer causing a decrease in tensile strength (Zinoviadou, 2013).

In (Plate VI, Fig. 4) it is evidenced that the elongation becomes lower as the moisture content decreases. This is logical as glassy biopolymers exhibit brittleness and fragility in severely low moisture conditions (Harris and Peleg, 1996). However, in moderate RH environments the partially plasticized matrix shows greater cohesiveness and stiffness.

These properties are observed due to the plasticizing effect of water and due to a better organization and higher molecular mobility within the matrix (Fontanet *et al.*, 1997).

At a moisture level of 11.3%, the control film showed a 6.5% of elongation. EB values of 4.4%, 5.7% and 12.5% were reported by Ozdemir and Floros, 2008; Osés *et al.*, 2009; Zinoviadou, 2013b respectively, when examined WPI films containing equivalent amounts of sorbitol in identical moisture contents. Addition of PG in the film matrix resulted to a great elongation increase, especially when PG was present in ratios 40:60 and 20:80 (WPI: PG), concluding that PG provided a plasticizing effect on the film matrix (Fig 3.4). Another research (Tonyali, Cikrikci and Halil, 2018) examined the effect of gum tragacanth (GT) on the mechanical properties on glycerol-plasticized WPI films, using homologous amounts of polymer and same RH conditions as the present study. When GT was added in an amount of 2% under 50% ± 2 RH, the tensile strength values were slightly higher (3.68 ± 0.25 MPa) compared to the results found in the present study (2.95 ± 0.4 MPa). Moreover, under the same RH conditions and concentrations of GT, the study revealed results on EB (69.25 ± 11%) higher than those of the present study (20.6 ± 2%). However, it should be noted that the aforementioned studies examined the tensile properties by equilibrating the samples for two days, compared to the present study which used a time period of ten days; short period of time may not be efficient for equilibrium to be reached, therefore the final results may be altered.

The effect of mesquite gum (MG) in sorbitol-plasticized WPI films was tested by Osés *et al.* (2009), following a method comparable to the present study. When a ratio of 50:50 (WPI: MG) was used under a moisture content of 9.8%, the study revealed a higher EB value (35.3%), compared to the present results (20.1%) which were observed under the same ratio (WPI: PG) and slightly lower moisture content (9%). Despite this, in the compared study the amount of gum was approximately double to the amount used in this study, therefore the increased amount of gum highly affected percentage of elongation. Comparing the 20:80 (WPI: PG) ratio of this study, in which the amount of gum used was closer to the ratio of the above study, the EB was found to be significantly higher (49%). Moreover, by contrasting the 50:50 (WPI: MG) values of the latter study under a moisture content 16.8% with the 20:80 (WPI: PG) of the present study under a slightly lower moisture content (12.8%), it was found that the EB results were equal. It may be concluded that it is of high importance for the moisture content of the samples to be calculated, as the real moisture within a film may greatly differ from the environment in which the films are stored, making thus results to be not comparable. A recent study examined PG for its film forming ability and revealed that composite of SFPG (3%) and glycerol (4%) produced a brittle film. Nonetheless, addition of $CaCl_2$ and rise of pH to 8 significantly improved flexibility, tensile strength and maximum elongation of the film (Dabestani *et al.*, 2018).

Conclusions And Recommendations For Further Research

The results of this study demonstrated that WVP was significantly increased when PG was added in ratios 60:40, 50:50, 40:60, and increased further in the ratio of 20:80 (WPI: PG). No great influence on moisture sorption isotherms was displayed when PG was present in the film matrix. PG caused a noticeable decrease on the tensile properties when added in a concentration of 80:20 (WPI: PG), however further addition of PG did not greatly decrease σ_{max}. A significantly higher elongation was observed when PG was present in ratios 40:60 and 20:80 (WPI: PG).

In summary, PG displayed considerable potentials to be used as a novel gum for edible films' formation. Incorporation of PG in the sorbitol-plasticized WPI films affected positively the elongation characteristics. However, improvement is needed for WVP to retain more stable values. This study suggests further research on the film-forming ability

of PG, such as the usage of irradiation or other methods which may improve the solubility of IFPG and may provide advanced properties in a film. Fractionation and separate examination of the soluble and insoluble fractions of PG may be also considered as the two fractions may display different characteristics when incorporated in a film matrix. Furthermore, as PG has not been widely examined for its film forming ability, the usage of different types and concentrations of polymers and plasticizers would provide information to be compared with the present study. By observing the fact that PG is widely used in countries of central Asia as a traditional remedy, its effect on the antimicrobial properties in edible films is suggested to be examined.

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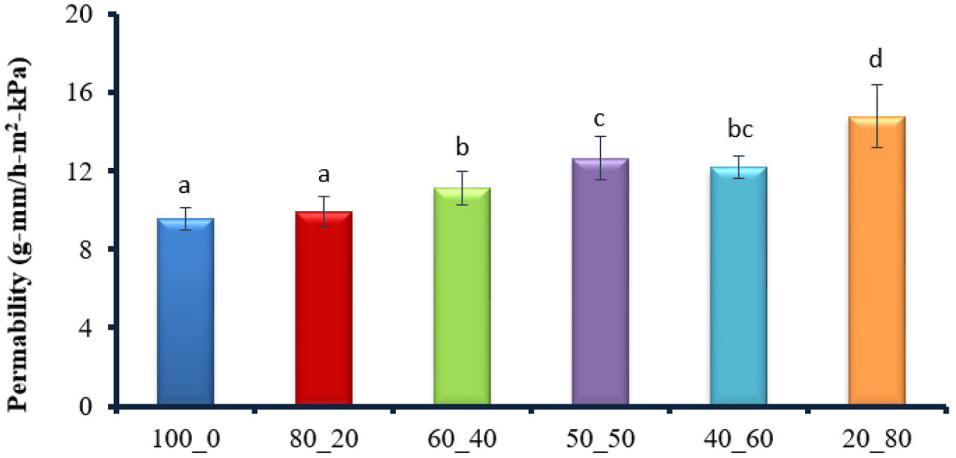


Fig. 1 Effect of PG concentration on the water vapour permeability of sorbitol-plasticized WPI films. Horizontal axis represents WPI: PG ratios (80:20, 60:40, 50:50, 40:60 and 20:80). Different letters indicate significant differences ($p < 0.05$).

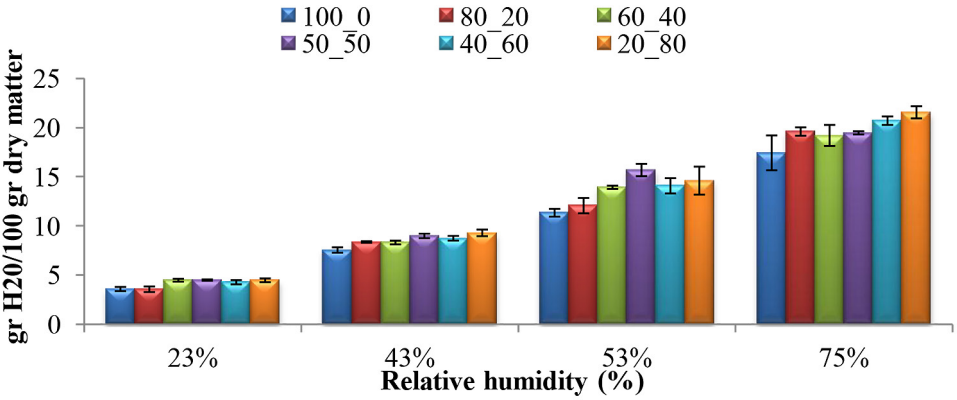


Fig. 2. Effect of PG concentration on the moisture sorption isotherms of sorbitol-plasticized WPI films. Letters represent: A: 100:0, B: 80:20, C: 60:40, D: 50:50, E: 40:60, F: 20:80 (WPI: PG). Error bars show standard deviation.

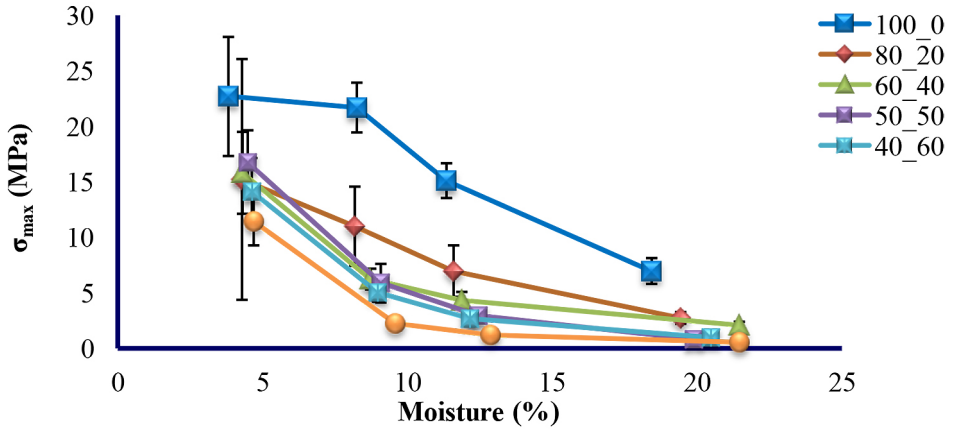


Fig. 3 Effect PG concentration (80:20, 60:40, 50:50, 40:60 and 20:80 WPI: PG) and moisture content on tensile strength (σmax) on sorbitol-plasticized WPI films. Error bars show standard deviation.

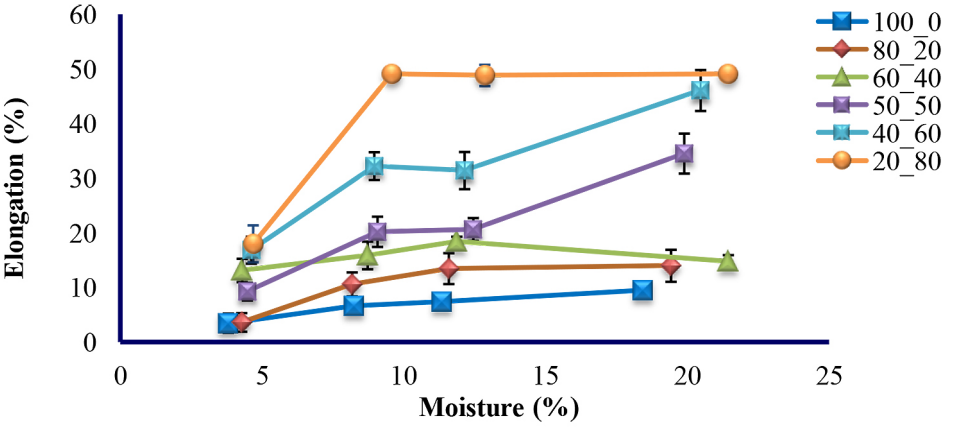


Fig. 4 Effect PG concentration (80:20, 60:40, 50:50, 40:60 and 20:80 WPI: PG) and moisture content on % elongation at break on sorbitol-plasticized WPI films. Error bars show standard deviation.