

Optimization of edible *alyssum homalocarpum* films for physical and mechanical properties

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Abstract

The effect of dry material, glycerol and ratio of water to seed in *alyssum homalocarpum* films on their thickness, transparency, moisture content, tensile strength, elongation and water vapor permeability was investigated using mixture response surface methods. Dry material and glycerol were important factors influencing tensile strength, elongation and water vapor permeability. Ratio of water to seed had little effect on physical and mechanical properties. The statistical methodology used determined the following optimal conditions for use in the casting process:

%20 glycerol, %0.3 dry material content and Ratio of water to seed was 2.5.

Keywords: Alyssum homalocarpum; Edible film; Mechanical properties; Water vapor permeability, Response surface.

Introduction

Food packaging plays a vital role in the quality of food products by providing protection from environmental, chemical, and physical challenges and preserving food throughout the distribution chain (Risch 2009). Packaging is used for several purposes (Berger 2002):

- Containment or agglomeration-Contain products, defining the amount the consumer will purchase and facilitate transportation and storing of products
- Physical and barrier protection- protects products from contamination, from environmental damage (mechanical shock, vibration, electrostatic discharge, compression,

temperature) and barrier from oxygen, water vapor and dust

- Information transmission-Packages and labels communicate how to use, transport, recycle, or dispose of the package or product and carry information and colorful designs that make attractive displays.

Plastic is the newest packaging material in comparison with metal, glass, and paper and they have become very important materials and a wide variety of plastics have been developed over the past 170 years (Brown 1992). The main problem with plastic (besides there being so much of it) is that it doesn't biodegrade and natural process can't break it down. Experts point out that the durability that makes plastic so useful to humans also makes it quite harmful to nature. Also the small bits of plastic (residual monomer and components in plastics, including stabilizers, plasticizers, and condensation components) can get sucked up by filter feeders and damage their bodies or other marine animals eat the plastic, which can poison them or lead to deadly blockages (Marsh and Bugusu 2007; Thompson et al. 2009). Therefore plastics are a problem with a very negative social and environmental impact. Some researchers believe that one way to deal with this problem is to use biodegradable/edible films (Fomin and Guzeev 2001). Edible films are defined as a thin layer of material which can be consumed and provides a barrier to moisture, oxygen and aroma movement for the food (Guilbert et al. 1995). Edible films can be produced from materials with film forming ability and can be classified into three categories: hydrocolloids

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(such as proteins, polysaccharides, and alginate), lipids (such as fatty acids, acylglycerol, waxes) and composites (Donhowe and Fennema 1993).

Among these materials polysaccharides from different sources have been extensively employed because of their relative abundance, good film-forming ability and nutritional qualities. Several studies have reported the use of polysaccharides from different sources (starch, non-starch carbohydrates, gums, and fibers) to prepare films with extensively employed (Chapman and Potter 2004).

Alyssum is a genus of about 100–170 species of flowering plants in the family Brassicaceae, native to Iran. The seeds are known to contain a large amount of mucilaginous substance and have been used as a traditional medicine in Iran. *Alyssum homalocarpum* comprises annual herbaceous plants clothed with stellate, white hairs, growing to 10–100 cm tall, with oblanceolate, or oblong-linear leaves and white flowers (Koocheki et al. 2010). In addition, these seeds have viscoelastic features and can use as gelling agents, thickening agents and emulsifiers.

The association among the polymers can be achieved through blending, laminating or coating with other polymers with desirable properties. Blending is an easier and more effective way to prepare compatible multiphase polymeric materials. Thus, the objective of this study is to investigate the influence of dry material content, glycerol and ratio of water to seed on mechanical and barrier properties, of *alyssum homalocarpum* -based films or coatings, as well as to evaluate their compatibility.

Materials and methods

Materials

Alyssum homalocarpum seeds were provided from a local market in the province of Shiraz in Iran. Glycerol was purchased from ACROS Co

(England). All other chemicals used were of analytical grade or the highest grade available.

Preparation of *alyssum homalocarpum* Solution

The *alyssum homalocarpum* seeds was cleaned and washed three times for 15 min using ethanol (%90). This process can remove all dust and pollution in the *alyssum homalocarpum* seeds. The seeds were then dried at 25°C in outdoors (out of direct sunlight) for 24 h at atmospheric pressure. The

Primary mixture film was prepared by dissolving some seeds in 100 mL distilled water using a mixer (IKA-RCT basic, IKA, Staufen, Germany) at a constant speed of 450 rpm at 25C for 30 min. Then, it was mixed in a stronger mixer (Sunny model, SFP, Sunny Electronics, Melbourne, Australia) for 5 min at 2000 rpm to completely separated gum and seed.

The nonsoluble portions of the *alyssum homalocarpum* seeds was separated from the transparent surface portion (as final solution) using a centrifuge at 2,500×g and 25C for 5 min, and the content of the dry material in the final solution was determined. Film was casted by vaporizing added solvent, and then its physical and chemical properties were studied.

Film thickness

Thickness of the films was measured with a digital micrometer (Mitutoyo 689037, Japan) to the nearest 0.001 mm in three random locations for each film and an average value was calculated. The average values were used in calculations for tensile properties and WVP tests.

Moisture content

The prepared film samples (3×3 cm² and conditioned at 25°C, RH = 53% for 48 h) were dried in an oven (Blue M Electric Co., Blue Island, IL) at 105°C until constant weight was obtained.

The moisture content was reported as the difference between the initial and final dry matter with respect to the initial dry matter. Three replications of each film treatment were used for calculating the moisture content.

Transparency

Transparency (low opacity) of the films was determined using a LUX METER (Testo 540 AG, Germany) for five randomly chosen portions of film. The average values for these five portions were used to identify the other physical and mechanical properties of the film.

Solubility

Film samples were dried at 105 °C for 5 h in a laboratory oven (Blue M Electric Co., Blue Island, IL), and weighed to determine the initial dry weight. Dried samples were placed in 50 mL of distilled water for 4 h under constant stirring conditions. After 4 h, they were removed from the water using a filter, dried for 5 h at 105°C, and weighed. Then the following formula (Eq. (1)) used to calculate the dissolved portion of dried film in water:

$$\text{Solubility (\%)} = [(T1-T2)/T1] \times 100 \quad (1)$$

Where T1 is initial dried film and T2 is insoluble dried film in water. The test had at least three repetitions for each film sample.

Mechanical properties

Tensile strength (TS), elongation at break (E%) of the film were determined at 25°C using a texture analyser (TA-XT2, Testometric Co. Ltd, Rochdale, England) according to ASTM standard method (ASTM D882 2012). The films were previously conditioned at room temperature at 53% RH in desiccators with saturated magnesium nitrate at 25°C for 48 h prior to testing. For the TS and E% determinations rectangular films strips of 100×10 mm were mounted to a self-aligning grip with 2 movable grips. The initial grip separation and cross-head speed were set to 50

mm and 30 mm/min, respectively. Five specimens from each film were tested from a minimum of three films per sample.

Water vapor permeability

A modified ASTM method (ASTM E96/E96M 2013) expressed by Hosseini et al. (2009) (Hosseini et al. 2009) was used to determine gravimetrically water vapor permeability (WVP) of the samples. Prior to the test, the films were placed in a chamber maintained at room temperature for 24h at 53% RH, to ensure equilibrium condition. After that, film specimens were sealed using parafilm and melted paraffin on glass container (5 cm diameter). To keep the gradient RH of 75% moving through the film, calcium chloride (0% RH) was used inside the glass containers and a saturated solution of sodium chloride (75% RH) in the desiccator. This difference in RH corresponds to a driving force of 1753.55 Pa, expressed as the water vapor partial pressure. Top side of film was facing towards the high or low RH environment during the test. Water vapor transport was determined from the weight gain of the permeation cell. Changes in the weight of the cell were recorded to the nearest 0.1 mg and plotted as a function of time. Linear regression was used to fit the data, weight vs. time, and to calculate the slope of the resulting straight line in gs^{-1} . The rate of vapor transfer (WVTR) was obtained by dividing the slope value (gs^{-1}) by the film surface (m^2). WVP was obtained by multiplying WVTR by the film thickness and dividing by the pressure difference between the relative humidity of the cells and the desiccator and calculated as:

$$\text{WVP} = [(WVTR \times d) / \Delta P] \quad (2)$$

Where d is the thickness of film sample (mm) and ΔP is difference of partial pressure (Pa). WVP was measurements of three replicated samples for each type of film.

Statistical analysis

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for designing experiments, building models through regression and evaluating the effects of multiple parameters and their interactions for responses (Myers and Anderson-Cook 2009). The analysis of variance (ANOVA) and response surface regression procedure of SPSS statistical software (v. 21) were used to analyze the average data from triplicate experiments at a confidence interval of 95 %. Experimental data were fitted to the following second-order polynomial equation to

all dependent Y variables (thickness, transparency, tensile strength, elongation, moisture content and water vapor permeability):

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 \quad (3)$$

Where β_0 is offset term, β_1 , β_2 and β_3 are the regression coefficients for linear effect terms, β_{11} , β_{22} and β_{33} are interaction effects. In this model, x_1 , x_2 and x_3 are the independent variables, namely dry material content, glycerol concentration and ratio of water to seed (Table 1).

Table 1. Experimental design for the *alyssum homalocarpum* films tests

| Blends | Composition | | |
|--------|--------------|----------|------------------------|
| | Dry material | Glycerol | Ratio of water to seed |
| 1 | 0.40 | 30.00 | 2.50 |
| 2 | 0.23 | 30.00 | 2.50 |
| 3 | 0.40 | 30.00 | 1.09 |
| 4 | 0.40 | 30.00 | 2.50 |
| 5 | 0.40 | 30.00 | 2.50 |
| 6 | 0.30 | 15.00 | 1.66 |
| 7 | 0.40 | 30.00 | 2.50 |
| 8 | 0.40 | 55.23 | 2.50 |
| 9 | 0.40 | 30.00 | 2.50 |
| 10 | 0.40 | 4.77 | 2.50 |
| 11 | 0.40 | 30.00 | 2.50 |
| 12 | 0.50 | 15.00 | 1.66 |
| 13 | 0.50 | 45.00 | 1.66 |
| 14 | 0.50 | 45.00 | 3.33 |
| 15 | 0.50 | 15.00 | 3.33 |
| 16 | 0.30 | 45.00 | 3.33 |
| 17 | 0.30 | 45.00 | 1.66 |
| 18 | 0.30 | 15.00 | 3.33 |
| 19 | 0.57 | 30.00 | 2.50 |
| 20 | 0.40 | 30.00 | 3.90 |

Results and discussion

Thickness

The ANOVA for the coded mathematical model for thickness indicated that the model was statistically significant ($p < 0.05$). The coded first-order model (linear) (Eq. (4)) for this variable was as follows:

$$Y_1 = 0.040 + 4.437 \times 10^{-3}x_1 + 2.549 \times 10^{-3}x_2 \quad (4)$$

According to Eq. (4), thickness was significantly affected by the dry material content and glycerol concentration (x_1 , x_2 ; $p < 0.05$) (Fig 1). The average thickness of *alyssum homalocarpum*-based films was 0.04 ± 0.004 mm.

Transparency

Transparency of films is of importance when they used as packaging materials (Yang and Paulson 2000). Visual characteristics of biodegradable films such as gloss, color, and

transparency can affect consumer acceptability and even food quality.

The average transparency of *alyssum homalocarpum*-based films was %94.97±1.044. The ANOVA for the coded mathematical model for thickness indicated that the model was statistically significant ($p<0.05$). The coded two-order model (quadratic) (Eq. (5)) for this variable was as follows:

$$Y_2 = 95.41 - 0.85x_1 + 0.58x_2 - 0.55x_3^2 \quad (5)$$

Thickness of the *alyssum homalocarpum* films increased significantly ($p<0.05$) with the addition of glycerol, as shown in Fig 2 but there was a negative correlation between the transparency with dry material content and ratio of water to seed.

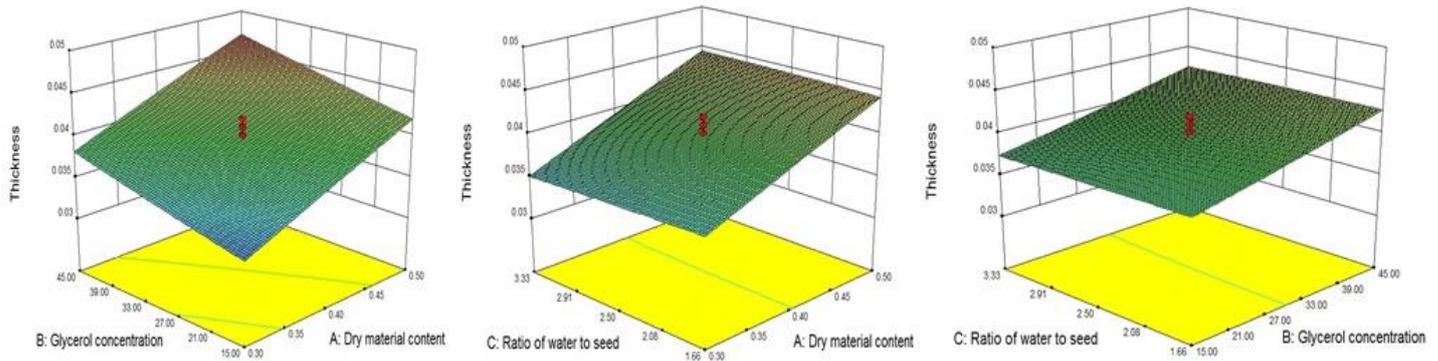


Fig 1. Response surface plots showing the effect of dry material content, glycerol and ratio of water to seed on thickness

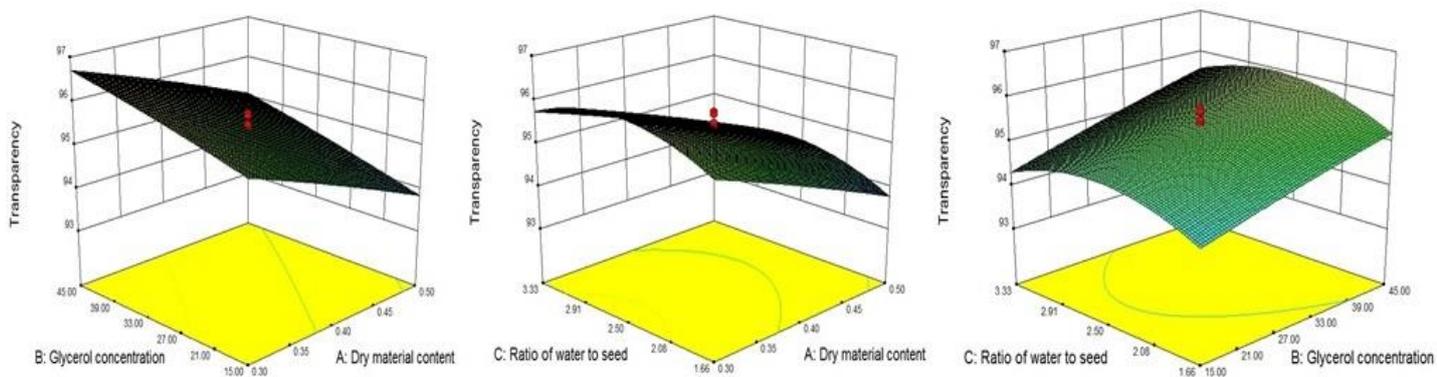


Fig 2. Response surface plots showing the effect of dry material content, glycerol and ratio of water to seed on transparency

Moisture content

As for the thickness and transparency, a regression analysis ($p<0.05$) was also performed to obtain a first-order model equation (linear) (Eq. (6)) for transparency as a function of the dry material content (x_1) and glycerol concentration (x_2).

$$Y_3 = \text{Moisture content} = 18.77 + 0.59x_1 + 0.91x_2 \quad (6)$$

The average moisture content of *alyssum homalocarpum*-based films was %18.75±0.92.

Mechanical properties of the films

Studying the mechanical properties of an edible film is relevant in order to predict its behavior when it has been applied to a food product. Tensile strength (resistance to elongation) and elongation at break (capacity for stretching) very useful parameters for describing the mechanical properties of a film, and are closely related with its internal structure (Falguera et al. 2011). The tensile strength and elongation of

alyssum homalocarpum based films are shown in Fig 4 and 5.

According to the results of ANOVA (Table 2), the calculated ($p < 0.05$) coded mathematical model for tensile strength (TS) and elongation at break (E%), represented by Eq.(7) and Eq.(8), allowing for plotting of the response surfaces.

$$Y_4 = 13.37 + 4.44x_1 - 2.36x_2 \quad (7)$$

$$Y_5 = 40.09 - 12.95x_1 + 29.18x_2 - 3.75x_1x_2 + 4.33x_2^2 - 3.29x_3^2 \quad (8)$$

According to Eq. (7), the most relevant variable with respect to TS, was the dry material content, but there was a negative correlation between the tensile strength and glycerol. According to Eq. (8), E% was significantly affected by glycerol but unlike the tensile strength, there was a negative correlation between the elongation at break with dry material content and ratio of water to seed.

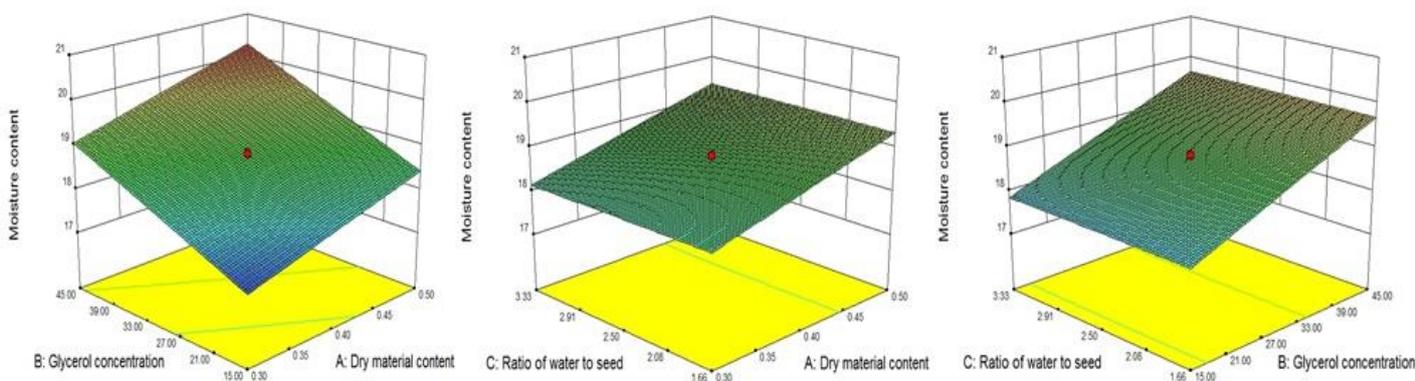


Fig 3. Response surface plots showing the effect of dry material content, glycerol and ratio of water to seed on moisture content

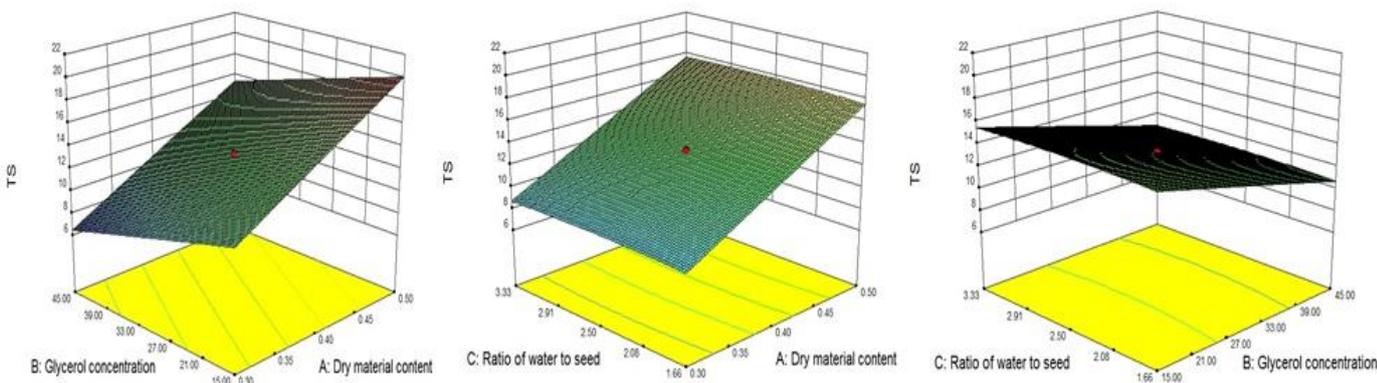


Fig 4. Response surface plots showing the effect of dry material content, glycerol and ratio of water to seed on tensile strength

Water vapor permeability

The effect of dry material content, glycerol concentration and ratio of water to seed on water vapor permeability is shown in Fig.6

WVP values of the different films with varying glycerol, dry material content and ratio of water to seed contents are given in Table 1. A regression analysis ($p < 0.05$) was also

performed to obtain a two-order model equation (quadratic) (Eq. (9)) for WVP as a function of the dry material content (x_1), glycerol concentration (x_2) and ratio of water to seed (x_3):

$$Y_6 = \text{WVP} = 14.85 - 2.45x_1 + 2.42x_2 - 1.26x_3 - 1.75x_1^2 \quad (9)$$

ANOVA results indicated that there was a significant effect of glycerol, dry material content and ratio of water to seed on water vapor permeability ($p < 0.05$).

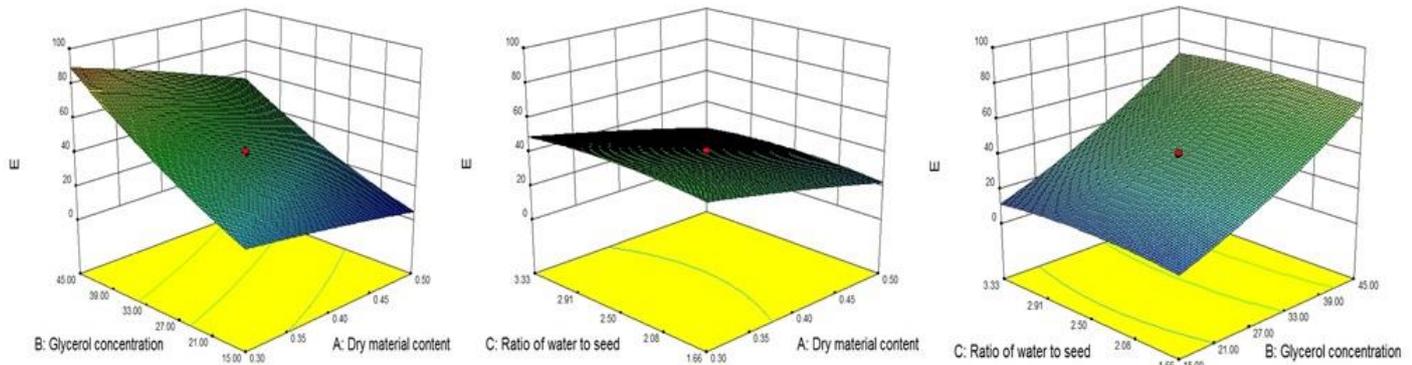


Fig 5. Response surface plots showing the effect of dry material content, glycerol and ratio of water to seed on elongation at break

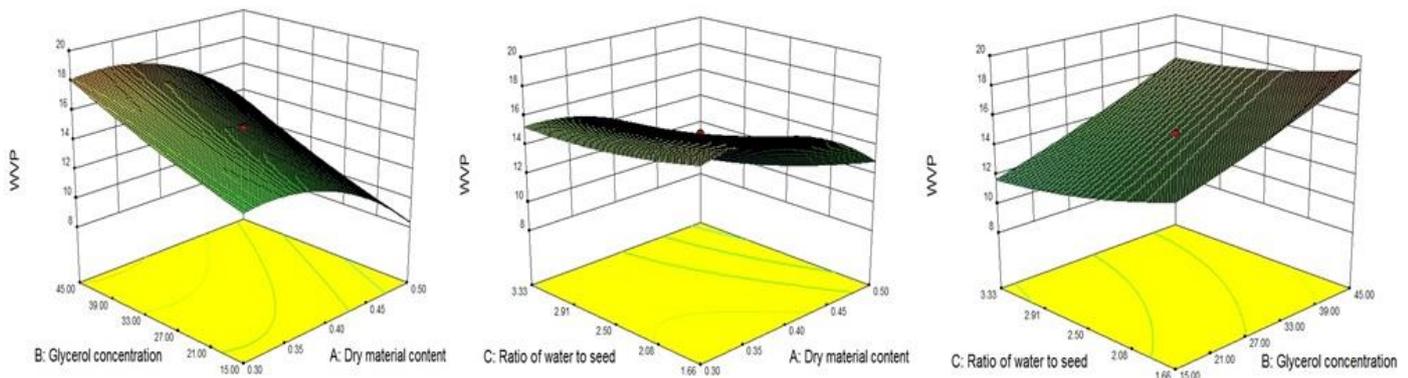


Fig 6. Response surface plots showing the effect of dry material content, glycerol and ratio of water to seed on WVP

Transparent, flexible, thin and homogeneous films were obtained from alyssum homalocarpum blended with glycerol. Visually, all the blended films had a slightly yellow appearance. The two sides of film had different appearances; the backside, which was in contact with the plate, looked homogeneous, while the top side did not.

Considering the hydrophilic nature of this polymer, increasing the dry material content increased the intermolecular bonds in the polymer matrix. Adding of glycerol and dry material content caused an increase in the

availability of hydroxyl groups and limiting polysaccharide-water interactions by the addition of hydrogen bonding and the formation of covalent bonds between the functional groups of alyssum homalocarpum chains, and resulting in an increase in the films thickness (Ekrami and Emam-Djomeh 2013). Similar research performed on starch and psyllium seed based polysaccharides confirmed the effect of dry material concentration and an increase in glycerol on the thickness of the film (Ahmadi et al. 2012; Godbillot et al. 2006).

Table 2. Regression coefficients and significant regression models for thickness, transparency, tensile strength, elongation, moisture content and water vapor permeability

| Coefficient | Thickness | Transparency | Moisture content | Tensile strength | Elongation | WVP |
|---|-------------------------|--------------|-------------------------|------------------|------------|-----------------------|
| β_0 | 0.040 | 95.41 | 18.77 | 13.37 | 40.09 | 14.08 |
| Linear | | | | | | |
| β_1 (Dry material) | $4.437 \times 10^{-3*}$ | -0.85^* | 0.59^* | 4.44^* | -12.95^* | -2.53 |
| β_2 (Glycerol) | $2.549 \times 10^{-3*}$ | 0.58^* | 0.91^* | -2.36^* | 29.18^* | 3.40 |
| β_3 (Ratio of water to seed) | -1.231×10^{-4} | 0.14 | -5.392×10^{-3} | 0.044 | 0.54 | -0.22 |
| Quadratic | | | | | | |
| β_{11} (Dry material \times Dry material) | -4.941×10^{-5} | -0.041 | -5.565×10^{-3} | -0.030 | -0.19 | -1.18 |
| β_{22} (Glycerol \times Glycerol) | 1.27×10^{-4} | -0.043 | -3.797×10^{-3} | -0.024 | 4.33 | 4.45 |
| β_{33} (Ratio of water to seed \times Ratio of water to seed) | -4.941×10^{-5} | -0.55 | -5.565×10^{-3} | -0.19 | -3.29 | -0.97 |
| Cross Product | | | | | | |
| β_{12} (Dry material \times Glycerol) | 0 | 0.046 | 0.015 | 0.029 | -3.75 | -7.5×10^{-3} |
| β_{13} (Dry material \times Ratio of water to seed) | 2.5×10^{-4} | 0.049 | 0.01 | 0.014 | -0.087 | -0.015 |
| β_{23} (Glycerol \times Ratio of water to seed) | 0 | 0.059 | -1×10^{-2} | 0.019 | 0.8 | 0.11 |
| C.V% | 3.29 | 0.42 | 0.58 | 3.73 | 11.02 | 36.32 |
| R-Squared | 0.9538 | 0.92 | 0.99 | 0.99 | 0.98 | 0.64 |

CV=coefficient of variance

Table 3. Responses of thickness, transparency, moisture content, tensile strength, elongation and water vapor permeability

| Blends | Thickness (mm) | Transparency (%) | Moisture content (%) | Tensile strength (MPa) | Elongation (%) | WVP |
|--------|----------------|------------------|----------------------|------------------------|----------------|-------|
| 1 | 0.04 | 95.34 | 18.75 | 13.37 | 39.27 | 13.9 |
| 2 | 0.031 | 97.16 | 17.66 | 5.16 | 67.23 | 18.78 |
| 3 | 0.04 | 93.61 | 18.76 | 12.75 | 29.45 | 14.73 |
| 4 | 0.039 | 95.42 | 18.69 | 13.32 | 40.14 | 13.86 |
| 5 | 0.04 | 95.68 | 18.7 | 13.35 | 38.21 | 13.92 |
| 6 | 0.034 | 94.86 | 17.38 | 11.16 | 17.15 | 9.9 |
| 7 | 0.038 | 95.5 | 18.82 | 13.39 | 41.09 | 13.87 |
| 8 | 0.045 | 96.51 | 20.42 | 8.97 | 104.4 | 27.59 |
| 9 | 0.041 | 95.76 | 18.86 | 13.34 | 40.38 | 13.9 |
| 10 | 0.035 | 94.49 | 17.09 | 17.75 | 3.26 | 32.16 |
| 11 | 0.042 | 94.67 | 18.79 | 13.4 | 40.95 | 13.92 |
| 12 | 0.042 | 93.29 | 18.42 | 19.39 | 6.17 | 5.58 |
| 13 | 0.046 | 94.26 | 20.18 | 14.99 | 52.33 | 18.68 |
| 14 | 0.047 | 94.69 | 20.16 | 15.12 | 55.1 | 18.48 |
| 15 | 0.042 | 93.22 | 18.46 | 19.36 | 2.03 | 4.56 |
| 16 | 0.038 | 95.88 | 19.02 | 6.72 | 81.43 | 22.89 |
| 17 | 0.039 | 95.91 | 19.06 | 6.73 | 82.03 | 23.43 |
| 18 | 0.034 | 94.86 | 17.36 | 11.16 | 17.08 | 9.34 |
| 19 | 0.048 | 93.85 | 19.84 | 21.53 | 10.77 | 9.11 |
| 20 | 0.039 | 94.52 | 18.74 | 13.05 | 35.07 | 14.33 |

The effect of dry material content and glycerol concentration on thickness is shown in Fig.1. The decrease in transparency by increasing the dry material content may be related to the polymer chain intervals and thickness (Ekrami and Emam-Djomeh 2013). Increasing the dry material increases the number of established bonds and the thickness of the *alyssum*

homalocarpum-based films and light penetration then decreases, decreasing the sample transparency. The effect of dry material content, glycerol concentration and ratio of water to seed on transparency is shown in Fig.2. Also when glycerol concentration increased, the film properties can change due to forming glycerol-polymer and glycerol-water

interactions (Osés et al. 2009). Probably increasing the dry material increases the number of established bonds between water and polymer and due to increasing of the water holding capacity (Ahmadi et al. 2012). The effect of dry material content and glycerol concentration on moisture content is shown in Fig.3.

The increasing tensile strength values of the blended films, with the dry material concentration increasing, are attributable to the formation of intermolecular bonds in backbone of *alyssum homalocarpum* films. But there was a negative correlation between the tensile strength and glycerol. Glycerol, as a plasticizer, reduces the intermolecular bonds between the polymer chains. Based on these results, it can be concluded that increasing of glycerol concentration in *alyssum homalocarpum* films improved film extensibility and reduced its resistance. Probably increasing of glycerol concentration in *alyssum homalocarpum* films, results in penetration of small molecular glycerol in the polymer matrix and reduction of inter-chain interactions. Therefore, a substantial increase in orientation and partial mobility of polymer chains, cause the extensibility of resulting films to be improved and the mechanical strength to be diminished (Yang and Paulson 2000). The effect of dry material content and glycerol concentration on tensile strength and elongation at break is shown in Fig.4 and 5 respectively.

Generally, water vapor transmission through a hydrophilic film depends on both the diffusivity and solubility of water molecules in the film matrix (Gontard and Guilbert 1994). A decrease in the inter chain space due to inclusion of dry material content between the polymer chains may less water vapor diffusivity through the film. The high hydrophilicity of glycerol molecules, which favors the adsorption of water molecules, could also contribute to the increase in the WVP of the film. Additionally, at the

high glycerol concentration, glycerol could aggregate with itself to open the polymer structure, enhancing the water permeability of the film still further.

Conclusion

Alyssum homalocarpum has good potential for forming edible film; however, the addition of glycerol as a softener is required to produce an optimal product. Glycerol increases flexibility and homogeneity and significantly affects the mechanical and physical properties of the *alyssum homalocarpum* film.

Alyssum homalocarpum appears to be a very interesting raw material for the preparation of edible films and coatings. The process developed here produced colorless films with good mechanical properties and excellent barrier properties. As usual, the glycerol content was the most important parameter influencing the mechanical properties, due to its plasticizing effects on the biopolymer matrix, followed by the effects of the dry material content value. The statistical methodology used determined the following optimal conditions for use in the casting process:

%20 glycerol, %0.3 dry material content and Ratio of water to seed was 2.5.

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