Electronic Journal of Biotechnology 55 (2022) 27–39

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage:

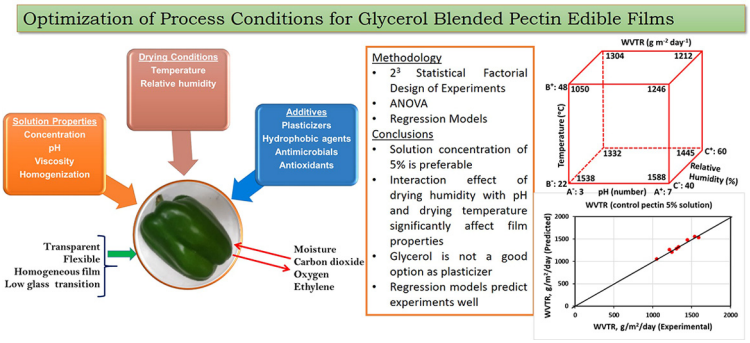
Research Article

Optimization of process conditions for the development of pectin and glycerol based edible films: Statistical design of experiments

Shumyla Mehraj, Yamini Sudha Sistla *

Department of Chemical Engineering, Shiv Nadar University, Gautam Buddha Nagar-201314, India

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 May 2021
Accepted 12 November 2021
Available online 18 November 2021

Keywords:
Biodegradable films
Biopolymers
Coatings
Edible films
Films
Food packaging
Glycerol
Pectin
Regression model
Statistical design
Water Vapor Transmission Rate

ABSTRACT

Background: Biopolymer based edible films have emerged as potential alternatives for conventional plastics in food packaging industry. The properties such as thickness, water vapour transmission rate (WVTR) and transparency of these films would be significantly influenced by the solution components and concentration and process conditions (pH, temperature and relative humidity of drying).

Results: Control and glycerol blended pectin films were developed as per 2^3 (two-level three-factor) factorial design of experiments by varying glycerol fraction (25% and 40% w/w) and solution concentration (3% and 5% w/v). The films made from 5% solution showed good moisture barrier properties. Glycerol addition reduced the moisture barrier capability of the films compared to control pectin films. Statistical analysis suggests that, the solution pH and drying temperature considerably affect film properties while the effect of relative humidity of drying is not evident enough. However, the interaction effect of relative humidity (H) with the pH and temperature appeared significant. Regression models were fitted to the data by considering the main and interaction effects, which were significantly affecting a particular property.

Conclusions: Detailed analysis reveals that for obtaining pectin based films with less thickness, low WVTR and high transparency, the optimal conditions preferred are low pH = 3, high T = 48°C and low to medium humidity of drying (H = 40–50%). The fitted regression models were statistically significant at 90% confidence level, pass Lack-of-fit analysis and are adequate to describe the effects of different factors on the targeted film properties.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso

* Corresponding author.
E-mail address: yamini.sistla@snu.edu.in (Y.S. Sistla).

https://doi.org/10.1016/j.ejbt.2021.11.004

How to cite: Mehraj S, Sistla YS. Optimization of process conditions for the development of pectin and glycerol based edible films: statistical design of experiments. Electron J Biotechnol 2022;55. https://doi.org/10.1016/j.ejbt.2021.11.004

1. Introduction

The growing demand for fresh and processed food having high quality and extended shelf life leads to a significant growth in food packaging industry [1,2]. The global food packaging market is expected to reach around USD 650 billion by 2025. Conventional coatings and films for food packaging are based on synthetic petroleum-based polymers. These synthetic polymers are harmful for human consumption. Furthermore, due to lack of proper recycling methods, these packaging materials, after usage, end up in landfills, beaches, rivers and oceans and cause nano- and micro level contamination for the humans and environment. The environmental concerns coupled with consumer awareness for sustainable alternatives have driven the research towards edible/ biodegradable coatings and packaging films. The edible/biodegradable packaging materials are based on bio-polymers derived from agricultural waste and other natural resources [3,4,5]. The main advantage of edible coatings is that they are palatable and digestible in human body. However, biodegradable films need not be edible but should be degradable within at least 6 months under normal atmospheric conditions. Edible films majorly consist of at least one component capable of forming a continuous and cohesive matrix such as polymeric materials/hydrocolloids. Natural polymers based on carbohydrates, proteins, lipids and fats have the ability to form gels in water, which, upon drying can form a homogeneous film like structure [1,2,6]. Post harvesting, fruits and vegetables lose moisture through transpiration which lead to drying, loss of shine, freshness and aroma. Also, respiration of living cells triggered by oxygen, causes increased release of carbon dioxide (CO_2), ethylene and heat due to oxidation of sugars and other cell reactions. This ultimately leads to gradual maturation, loss of nutrients and eventual deterioration. Edible coatings and films can protect and improve shelf life and freshness of food by controlling the internal atmosphere by providing moisture barrier and facilitating equilibrium levels of oxygen and carbon dioxide, and improve the mechanical handling property [4,5,6,7,8]. However, additional properties such as transparency, high tensile strength, flexibility, ability to form very thin films, stability at cold storage and atmospheric conditions and easy biodegradability are also critical. They must also act as good carriers for various antioxidants, antimicrobials, vitamins and nutrients [9]. The appropriate bio-based materials for edible films are chosen based on factors such as availability, cost, moisture and gas barrier properties, mechanical properties, transparency and resistance to microorganisms. The edible films usually are composed of various biopolymers like carbohydrates, proteins and lipids with some amount of plasticizers to form flexible films and other additives such as binders, hydrophobic agents, fillers, antimicrobial agents [10,11]. Researchers have developed different formulations of edible films and coatings based on proteins, polysaccharides, starch and lipids to improve the shelf life of fruits and vegetables, meat, poultry and minimally processed foods [9,10,11,12,13,14,15]. It is ascertained that, lipid (e.g. fat) based films demonstrate good moisture (water vapour) barrier properties, polysaccharides can control the transmission of oxygen and other gases such as CO_2, while protein based films provide good mechanical stability [14]. Among carbohydrates, the class of polysaccharides are abundantly available from natural sources and have an attractive property of gel formation in water. Pectin is an anionic poly-saccharide which is usually present in many primary and middle lamella of plant cell walls. Some natural sources of pectin include, citrus peels, banana peel, mango peel, watermelon seed, sugar beet pulp and tomato pulp to name a few [16]. Chemically pectin is a long chain of Homogalacturonan (HG), Xylogalacturonan (XGA) Apiogalacturon and Rhamnogalacturonan (RGA) I & II. Among all the components, HG forms more than 65% of pectin whereas RGA I and II fraction is only 35% and rest contribute to 10 to 20% of the pectin [16,17]. The Homogalacturonan is composed of repeat units of (1 → 4)-α-D-galactouronic acid forming the backbone of pectin. The other sugar moieties XGA and RGA are considered as the hairy region of the pectin molecule. The galactouronic acid, based on the degree of esterification (DE > 50%), show good gelling properties [10,16,17]. Because of its good gelling and binding properties, we have selected pectin for the present study to develop edible films. Till date, most of the researchers have worked on formulations based on proteins, collagen, starch and cellulose. However, the literature on pectin based films is not ample. Pectin is of low cost and abundantly available in the tissues of fruits and vegetables which makes it sustainable option to make the edible coatings and films [18,19,20]. Yearly, around 50 mt of pectin is being produced worldwide to meet the demand of ∼150 mt [21]. Pectin based coatings are known for good oxygen and carbon dioxide barrier and aroma preservation properties along with their ability to retard lipid migration but with a limitation of being hydrophilic [20,22,23]. Chakravarthula et al. [24] have developed composite films based on pectin, alginate and whey protein in various concentrations. They reported that whey protein or alginate did not enhance the properties such as elongation modulus, water vapour permeability of the pectin films, while, whey protein provided slight plasticizing properties [24]. Cabello et al. [25] have studied the microstructural changes in pectin films with the addition of plasticizers such as glycerol and polyethylene glycol (PEG). They reported that glycerol acts as internal plasticizer while PEG acts as external plasticizer. Furthermore, glycerol reduces the water vapour barrier properties of pectin films while a high molecular weight PEG improves the water vapour barrier properties [25]. Mendes et al. [26] have reported that addition of cocoa butter to pectin improves the mechanical strength and thermal stability of the films along with improved transparency and reduced water vapour transmittance. Phuong et al. [27] have reported that the films with 50:50% of pectin and nano-chitosan have showed improved tensile strength and reduced moisture and gas permeability. Priyadarshi et al. [28] have reported that addition of 50% of pullulan to pectin has improved the water barrier properties and thermal stability of the films. Younis et al. [29] have reported that incorporating the chitosan/pectin nano-fibres into pectin films improved water-proof ability, thermal stability, break resistibility, stretch ability and UV blocking capacity.

The quality of an edible/bio-degradable film or coating depends on processing conditions (pH, temperature and relative humidity of drying), solution concentration, type of solvent, and type of additives (plasticizers, emulsifiers, cross-linking agents, antimicrobials and anti-oxidants) [6,9,30,31]. Research papers discussing in detail about the effect of process conditions on the properties of pectin based films are scarce. Therefore in the present study we have used statistical design of experiments to identify optimum

process conditions such as pH of solution, temperature and relative humidity of drying environment for developing pure pectin films and glycerol (plasticizer) blended pectin films having minimum thickness, more moisture barrier capability and more transparency.

2. Materials and methods

Chemical grade extra pure pectin was purchased from Loba Chemie Pvt. Ltd, Mumbai, India. The molecular weight of pectin was in the range of 30,000–100,000. The degree of esterification (DE) of pectin was 63–66% and methoxyl content was 6–10%. The other chemicals such as glycerol, sodium hydroxide buffer tablets and other required chemicals in analytical grade were purchased from Sigma Aldrich. Double distilled water was made in our laboratory.

2.1. Film preparation

All the films were prepared in a lab scale petri dish. In order to make 3% weight/volume (weight/volume is mentioned as w/v here after) of control pectin films, 3 g of pectin powder was dissolved in 100 mL of distilled water. The solution was homogenized for 20 min at a temperature of 50°C using a magnetic stirrer with heating arrangement. Then the solution was allowed to cool to room temperature. The pH of the resulting solution was measured and it would be usually acidic (pH = 2.8) because of the high acidic nature of pectin. Therefore, pH of the solution was adjusted to the required value by adding sodium hydroxide buffer tablets. After adjusting the pH, the solution was again heated to 50°C for 15 min. The hot solution was then homogenized using a Homogenizer (IKA T25 ULTRA-TURRAX) at an rpm of 4000–6000 to get the film forming solution. A 25–30 mL of this hot solution was poured into a 100 mm diameter petri dish and spread by quivering. The films were then dried by evaporating the solvent in a controlled environment of temperature and relative humidity in a Humidity chamber (NECSTAR NEC-HTC-150). The dried films were peeled off and stored in a desiccator. Same procedure was used for preparing control pectin films using 5% w/v (5 g pectin in 100 mL water) composition. Glycerol was used as a plasticizer to improve the flexibility and strength of pectin films. Glycerol blended pectin films (mentioned as "pectin + glycerol" here after) were prepared by adding 1 mL of glycerol (Density of glycerol is 1.26 g/mL) to the 3% and 5% w/v pectin solutions in water. The same experimental procedure stated above was used for preparing the glycerol blended pectin films. Consequently, four types of pectin based films were developed (1) control pectin films using 3% w/v solution (2) control pectin films using 5% w/v solution, (3) pectin + glycerol (P:G = 3.00:1.26 w/w) films using 3% w/v solution (4) pectin + glycerol (P:G = 5.00:1.26 w/w) films using 5% w/v solution. The prepared films were stored in a desiccator containing silica gel (0% relative humidity).

2.2. Characterization of the films

2.2.1. Moisture content and water solubility

The bound moisture content of the film was determined by keeping a 2 cm × 2 cm film in a hot air oven at a temperature of 100 ± 2°C for 6 h and then calculating the percent weight loss of the film compared to the initial weight. The moisture content was analysed by taking three samples from the same film to check the consistency.

Water solubility of the films was tested by soaking a 3 cm × 3 cm film in water for 2 h. The remaining film was taken out, dried and weighed. Percentage water solubility was then calculated using the formula

$$\%WS = \left(\frac{W_{initial} - W_{final}}{W_{initial}}\right) X100$$

where %WS is percent water solubility, $W_{initial}$ is the initial dry weight of the sample film, W_{final} is the final dry weight of the sample film.

2.2.2. Surface morphology analysis

The surface morphology of the films was analysed using a Polarizing microscope (Olympus BX53; Olympus Optical Co., Ltd) to evaluate the surface homogeneity and structure. Samples were observed in black and white and the images were recorded at 10x magnification. The analysis was conducted at room temperature. The analysis was performed at ten different positions on the film and repeated five times.

2.2.3. Thickness

Films were developed by pouring a 25–30 mL of the hot film forming solution into a 100 mm diameter petri dish and spread by quivering. The thickness of the film was measured using a Micrometre screw gauge with 0.001 mm least-count, at ten random positions on the film of 100 mm diameter.

2.2.4. Optical properties

Optical properties of edible films are essential sensory aspects for consumer acceptability of the films. Transparency of the film would improve overall acceptability of the film for packaging applications. Color/transparency analysis of the films was done using a Spectrophotometer (CHN SPEC & CS-580A) calibrated by keeping a transparent polymeric film on a white plate (L^* = 86.75, a^* = 0.93 and b^* = −1.03). Film color was measured by lightness/luminosity (L^*), chromaticity (a^*), chromaticity (b^*) on color scale. L^* values refer to the range from black (zero) to white (100), a^* ranging from green (negative) to red (positive) and b^* ranging from blue (negative) to yellow (positive). Five repetitive measurements were made for each sample at 5 different locations on the sample and the average of the values was considered. Total colour difference/transparency parameter (ΔE) and whiteness index (WI) were calculated as shown below:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

where, L^*, a^*, b^* are standard values of a transparent sheet kept on a white plate and L, a, b are the measured values of the samples. The standard values of a transparent film used as reference in the present study are L^* = 86.75, a^* = 0.93 and b^* = −1.03.

2.2.5. Water vapour transmission rate

Water vapour transmission rate (WVTR) analysis of the films was done at Northern India Textile Research Association (NITRA), Ghaziabad, India, as per the ASTM standard ASTM E96/E96M-05 (water method). A brief description of ASTM E96 method: A cup was filled with distilled water leaving a small gap (0.75″ to 0.25″) of air space between the specimen and the water. The cup was then sealed to prevent vapour loss except through the test sample. The atmospheric chamber was kept at a temperature of 32 ± 2°C and a relative humidity of 50 ± 2%. Air velocity was set in the range of 0.02–0.3 m/s. Initial weight of the apparatus was measured and noted followed by periodical readings of the weight of apparatus until results became linear. The WVTR of the samples in the present study is reported in the units of g m^{-2} day^{-1}. The

water vapour permeability (WVP) was calculated by the equation below. WVP in the present study is reported in the units of g m^{-1} s^{-1} Pa^{-1}.

$$WVP = WVTR * \frac{x}{p^{sat}(RH1 - RH2)}$$

where x is the film thickness in meters (m), p^{sat} is the saturated vapour pressure of water vapour at 32°C which is 4.75 kPa as per thermodynamic saturated steam tables [32]. (RH1- RH2) is the difference in relative humidity between the two sides of the film where, RH1 = 52% and RH2 = 0%.

2.3. Experimental design and statistical analysis

A two level 2^3 factorial design of experiments was used to understand the effect of three independent variables or factors such as pH of the solution (P), temperature (T) and relative humidity (H) at which the films were dried. Among various properties of the films studied, we have selected thickness (Th), Water Vapour Transmission Rate (WVTR) and transparency parameter (ΔE) as dependent variables or desired responses for the statistical analysis in order to optimize the process conditions [33]. In the two-level factorial design, the levels of the factors are generally called "low" and "high" levels and coded as (−1) and (+1), respectively. The ranges of the independent variables were chosen based on literature and preliminary screening of experimental observations conducted in our lab. The low and high levels of the independent variables considered for the present study are given in Table 1. Based on the sensitivity and complexity of the experiments and the results, Analysis of variance (ANOVA) at 90% confidence level was used to identify statistically significant differences between the mean results associated with each property and treatment.

Analysis of Main Effects and Interaction Effects: For a 2^3 factorial design, the main effects of different factors and their interaction effects (two-factor and three-factor interactions) on the responses are determined. For example, the main effect of a factor or an independent variable is defined as the change in response produced by a change in the level of that factor from the low level (−1) to the higher level (+1) averaged over the levels of other factors. The main and interaction effects are determined using the method of contrast coefficients, which is well described elsewhere [33]. For example, contrast coefficients for one set of data is provided in supporting information (Table S1).

Regression Models: The present study aimed to obtain optimal process conditions (three factors: P, T and H) to reduce the thickness (Th), lower the WVTR and increase transparency (low ΔE value) of the films. The first-order linear regression models with main factors and their interaction terms were fitted to the experimental data of the response variables, by neglecting the factors that are not affecting the response variables based on the analysis of the main and interaction effects.

$$Y_{predicted} = \beta_0 + \beta_1 P + \beta_2 T + \beta_3 H + \beta_{12} PT + \beta_{13} PH + \beta_{23} TH + \beta_{123} PTH$$

Table 1
Details of low and high levels of the independent variables or factors chosen.

	Levels	
	Low (−1)	High (+1)
Independent variables		
P: pH (number)	3	7
T: Temperature (°C)	22	48
H: Relative Humidity (%)	40	60
Desired Responses		
Th: Thickness (mm)	Minimize	
ΔE: Color transparency (%)	Maximize	
WVTR: Water vapor permeability (g/m^2/day)	Minimize	

where Y refers to the response variable (Thickness (Th) or WVTR (or) ΔE); β_0 is the intercept of the equation, and β_1 - β_{123} are the regression coefficients. P, T, and H are coded forms for the main factors: pH, Temperature and Relative Humidity. PT, PH and TH are two-factor interactions between respective two factors. PTH is three-factor interaction between P, T and H.

The intercept β_0 of the regression model is estimated by the average of all response variable observations. The regression coefficients β_1 - β_{123} are one-half the value of corresponding factor effect estimates [33].

Four different data sets were used for regression model fitting, which resulted in three response models for each data set (total 12 response models). The adequacy of all models was verified at 90% confidence level by applying lack-of-fit F-test and *p*-value [33].

3. Results and discussion

Four types of films were developed (1) control pectin films from 5% w/v solution (2) control pectin films from 3% w/v solution, (3) pectin + glycerol (P:G = 5:1.26 w/w) films from 5% w/v solution (4) pectin + glycerol (P:G = 3:1.26 w/w) films from 3% w/v solution. All the films were developed based on the 2^3 (two-level three-factors) full-factorial design of experiments. The experiments were conducted randomly to avoid any bias. The values of the responses that include: film thickness, WVTR, WVP, ΔE and WI are summarised in Table 2 and Table 3 respectively for the four film formulations mentioned above. The films were strong and flexible enough to peel off from the petri dish and to handle. All the films were stored in a desiccator to avoid any contamination from moisture and atmospheric gases. The thickness of the films was observed to be in the range of 0.12–0.28 mm, whereas the transparency ΔE of the films was in the range of 1.63–19.68. Where, ΔE = 1.63 refers to a more transparent film and ΔE = 19.68 refers to a less transparent film with reference to the standard used. It is important to note that the thickness and transparency need not to be directly proportional. Because, the film thickness depends on the concentration of the solution when the same amount of solution with different concentrations was poured in a petri dish of specific diameter. On the other hand, the transparency is more influenced by the microstructure, components and composition of polymer films rather than the thickness. For instance, the microstructure of a film is typically influenced by the pH of solution, properties of constituents of the film, solution concentration, degree of homogenization and drying conditions. Mean values of the four sets of films suggest that, the 5% solution films (both control pectin and pectin + glycerol) were relatively thick and less transparent compared to those prepared from 3% solution. The ΔE of 5% control pectin films was in the range of 3.39 ± 5.45 to 18.87 ± 5.45, while the ΔE of 3% control pectin films was in the range of 3.1 ± 2.39 to 10.1 ± 2.39 based on the process conditions. For a control pectin film formed using 3% solution, and dried at 50% relative humidity and 25°C, the ΔE reported by Chakravarthula et al. [24] was 11.3 ± 1.1. Sartori et al. [23] have developed pectin + glycerol films (4% w/w of pectin solution with the addition of glycerol at 1.5 w/w of pectin) dried at 40°C and 50% RH. The developed film had a thickness of 0.074 mm and a ΔE of 11.4 ± 0.8 [23]. The average ΔE of films from 5% solutions of both control pectin and pectin + glycerol reported in the present work (Table 2 and Table 3) match well with Chakravarthula et al. [24] and Sartori et al. [23]. Furthermore, the 3% solution films showed an average ΔE of 5.72 ± 2.39 for control pectin and 8.52 ± 5.25 for pectin + glycerol. This indicates that our films from 3% solution are more transparent compared to Chakravarthula et al. [24] and Sartori et al. [23]. The 32 films (control pectin & pectin + glycerol) reported in the present study showed WVTR in the range of 1050 g.

Table 2
The 2^3 factorial design matrix for control pectin films.

Run No	Factors			Responses for 5% w/v solution					Responses for 3% w/v solution				
	P	T (°C)	H (%)	Thickness (mm)	WVTR ($g/m^2/day$)	WVP (g/m s Pa)	ΔE	WI	Thickness (mm)	WVTR ($g/m^2/day$)	WVP (g/m s Pa)	Δ E	WI
1	3 (−1)	22 (−1)	40 (−1)	0.186	1538	1.34E-09	6.37	81.3	0.201	1905.6	1.79E-09	3.21	84.29
2	3	22	60 (+1)	0.152	1332	9.48E-10	8.72	80.33	0.092	2018.4	8.70E-10	4.78	83.28
3	3	48 (+1)	40	0.171	1050	8.41E-10	6.76	84.17	0.152	1135.2	8.08E-10	4.94	83.25
4	3	48	60	0.22	1304	1.34E-09	3.39	87.41	0.230	2105.1	2.26E-09	3.10	88.07
5	7 (+1)	22	60	0.194	1444.8	1.31E-09	13.9	75.5	0.107	2575.2	1.29E-09	8.70	80.01
6	7	22	40	0.135	1588.8	1.01E-09	13.16	76.73	0.083	1838.4	7.15E-10	10.10	79.48
7	7	48	40	0.244	1245.6	1.42E-09	18.87	70.3	0.021	1953.6	1.92E-10	7.02	87.85
8	7	48	60	0.19	1212.1	1.08E-09	18.8	74.68	0.093	1675.2	7.30E-10	7.43	84.07
			Mean	0.18	1339.4	1.16E-09	11.24	78.81	0.12	1900.8	1.08E-09	6.16	83.78
			Std. dev	0.006	18.47	3.21E-11	0.16	0.13	0.009	21.28	2.25E-11	0.38	0.32

Table 3
The 2^3 factorial design matrix for pectin + glycerol films.

Run No	Factors			Responses for 5% w/v solution					Responses for 3% w/v solution				
	P	T (°C)	H (%)	Thickness (mm)	WVTR ($g/m^2/day$)	WVP (g/m s Pa)	ΔE	WI	Thickness (mm)	WVTR ($g/m^2/day$)	WVP (g/m s Pa)	Δ E	WI
1	3 (−1)	22 (−1)	40 (−1)	0.252	2025.6	2.12E-09	16.55	72.74	0.174	2347.4	1.91E-09	6.55	80.77
2	3	22	60 (+1)	0.119	2234.4	1.24E-09	4.64	82.56	0.093	2623.2	1.14E-09	1.63	85.45
3	3	48 (+1)	40	0.192	1728.7	1.55E-09	6.76	84.17	0.174	2635.0	2.14E-09	6.46	83.89
4	3	48	60	0.263	1917.6	2.36E-09	4.17	85.49	0.240	2644.0	2.97E-09	4.11	89.27
5	7 (+1)	22	60	0.282	1951.2	2.57E-09	15.52	74.05	0.241	2364.3	2.66E-09	7.73	80.60
6	7	22	40	0.213	1809.6	1.81E-09	10.97	78.16	0.184	2364.5	2.03E-09	19.68	69.13
7	7	48	40	0.171	1291.2	1.03E-09	17.21	77.00	0.160	1956.0	1.46E-09	13.08	81.63
8	7	48	60	0.209	2198.4	2.15E-09	12.73	82.55	0.160	2006.0	1.50E-09	8.94	87.57
			Mean	0.212	1894.6	1.85E-09	11.07	79.59	0.178	2367.4	1.98E-09	8.52	82.28
			Std. Dev	0.002	47.51	6.16E-11	0.49	0.45	0.006	52.62	1.08E-10	0.47	0.49

$m^{-2}.day^{-1}$ to 2644 $g.m^{-2}.day^{-1}$ and WVP in the range of 1.92×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ to 2.97×10^{-9} $g.m^{-1}.s^{-1}.Pa^{-1}$. These WVP values are close to the available reported literature values. Solak et al. [34] have used apple pectin (DE = 56.9%) to develop films from 2.5% solution and dried at 35°C under vacuum. These films reportedly shown WVP of 1.19×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ which is very close to our values. Phuong Ngo et al. [27] have used pectin extracted from *Tiliacora triandra* (DE of 48%) using a 2% solution. These films reportedly showed WVP of 0.16×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ which is close to our values. Liu et al. [35] have developed high methoxy pectin films having WVP of 5.12×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ which is very close to our values. Chakravarthula et al. [24] have developed films from citrus peel extracted pectin which reportedly shown WVP of 0.2×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ which is in close correspondence with our values. Sartori et al. [23] have reported a WVP of 1.5×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ for low methoxy pectin films developed using 4% solution and is very close to our values. Younis et al. [29] have developed high methoxyl apple pectin films having WVP of 1.5×10^{-10} $g.m^{-1}.s^{-1}.Pa^{-1}$ which is very close to the values reported in the present work. Both the control pectin and pectin + glycerol films developed by us from 5% solution showed ~30% less WVTR compared to 3% solution films. The WVTR and WVP depends on factors such as the number of polar groups (hydroxyl groups) present on the surface of polymer network, thickness of the film and also microstructure of the film. The number of hydrophilic/polar groups depends on the degree of esterification (DE) of pectin. The DE of chemical grade pectin used in the present study was 63–66% and methoxyl content was in the range of 6–10%. The DE and methoxyl content of present study falls under medium range which gives gelling properties to pectin. At high degree of esterification, WVTR decreases due to increase in hydrophobic ester groups. This explanation justifies the relatively high WVTR shown by the chemical grade pectin we have used for our studies. The water transport in the polymer film usually is facilitated by sorption of water molecules and further migration via polar hydroxyl groups. When the films are made from same amount of sample in the same sized petri dish, the microstructure of the 5% films would be more compact compared to 3% films. This might have led to the decrease in WVTR of 5% films compared to 3% films. When compared at the same solution concentration, addition of glycerol did not seem to show any improvement in terms of thickness or transparency of the film. However, addition of glycerol increased the WVTR by 30–40% which is not an encouraging result. The increase in WVTR is due to the hygroscopic nature of glycerol. Therefore, although glycerol is a good plasticizer to give good tensile properties and flexibility to the pectin film, it increases the hydrophilicity of the pectin film and thus the WVTR [36]. A detailed statistical analysis of the effect of the three factors (P, T & H) on the three responses (thickness, WVTR and transparency) for all the four types of films is discussed in the Sections 3.3.1–3.3.4.

3.1. Moisture content and water solubility of the films

The bound moisture content of the films (both control pectin and pectin + glycerol films) was observed to be between 2% and 7%. The pectin + glycerol films showed relatively more bound moisture content than those from control pectin. The hygroscopic nature of glycerol favours the absorption of water molecules leading to formation of hydrogen bonds in the polymer film matrix and thus increases bound moisture percentage of the film.

All the pectin + glycerol films were completely soluble in water as per the test details mentioned in Section 2.2.1. However, the control pectin films were not completely soluble in water. Some portion of the film still left insoluble even after 2 h. The water solubility of control pectin films observed to be in the range of 65–75%.

3.2. Surface morphology of films

The micrographs of film surfaces evaluated by optical microscope (10× magnification), are shown in Fig. 1. The micrographs clearly show that all the films were homogenous, uniform and translucent. There were no agglomerations observed. The micrographs for control pectin films are consistent with those reported AFM and SEM micrographs elsewhere in the literature. The micrographs of control pectin films in Fig. 1a,b,c appear similar to the micrographs visualized using Atomic Force Microscope (AFM) by Sartori et al. [23]. The control pectin micrographs also look similar to the SEM images of control pectin films reported by Chakravarthula et al. [24], Phuong Ngo et al. [27], Priyadarshi et al. [28], and Younis et al. [29]. The morphology of the films was supported by the trends of transparency and WVTR, evidencing that the structural arrangement of the components in the film and processing/drying conditions affect the overall characteristics of the film. The surface morphology of the films formed at conditions of pH = 3, T = 22°C, RH = 40% and pH = 7, T = 22°C, RH = 40% seems to be similar. However, there is a visible difference in the morphology of films at pH = 7, T = 22°C, RH = 40% and pH = 7, T = 48°C, RH = 60%. This indicates that the drying conditions (temperature and relative humidity) can influence the surface morphology of films.

3.3. Main and interaction effects

3.3.1. Control pectin films made from 5% solution

The thickness, WVTR, WVP, transparency (ΔE) and whiteness index (WI) values of the 2^3 full factorial experimental design are summarised in Table 2. Standard deviation of the responses were calculated by repeating the run numbers 3 and 7 three times. Based on the repeated experiments 3 and 7, the standard deviation in thickness, WVTR and ΔE were 0.006, 18.47 and 0.16, respectively. The standardized effects of main factors, two- and three-factor interactions, mean sum of squares and the percentage contribution of each factor and factor combinations on the three response variables are shown in Table 4. Positive effect means, as the factor level increases, the response variable increases. Negative effect represents a decrease in response variable with an increase in the factor level.

Analysis for less thickness: the two-factor interactions PH, TH and three-factor interaction PTH show negative effects on thickness, while P, T, H and PT show positive effects. The main factor T and the three-factor interaction PTH followed by P and PT contribute more for the thickness of the film, while the main factor H and the other two-factor interactions PH and TH do not contribute significantly. The positive effect of T along with a significant contribution (36%) clearly indicates that the low temperature is favourable for getting thin films from 5% solution. However, the role of three-factor interaction PTH (55%) is also significant and shows positive effects on thickness indicating either a low to moderate P and a low to moderate H at a fixed low T. The next significant contribution (2%) comes from P, which has a positive effect, indicating that low pH is desirable to obtain thin films.

Analysis for low WVTR: The factors T, H, PT, PH and PTH show negative effects on WVTR, while the factors P and TH show positive effects. The main effects P and T and the two-factor interactions PH and TH and the three-factor interaction PTH show significant effect on the WVTR, while other factors do not seem to influence WVTR significantly. Temperature has highest influence (66%) and has a negative effect on WVTR. This indicates that a high temperature is favourable to form films with low WVTR from 5% solution. The next highest contributor is the two-factor interaction TH, which has a positive effect. This means the TH effect should be low. As it is already known that high T is favourable, it is the relative humidity (H), which has to be low to medium value in order to reduce the TH effect.

Analysis for high transparency: High transparency means low ΔE value. The factors H and TH show negative effects on the ΔE, while P, T, PT, PH, PTH show a positive effect. The ΔE is affected more by the main effects P, T and the two-factor interactions PT and TH and the three-factor interaction PTH, while the effect of other factors is insignificant. The highest contribution (82%) from pH (P) and a positive effect by P indicates that low pH is favourable to obtain transparent films (low ΔE value) from 5% solution. The next contributing factor is PT (12%) and has a positive effect on ΔE. In order to reduce the PT, with an already fixed low P, a low to high T are favourable to get transparent films.

The influence of two-factor interactions at the base level of the third factor and the combined effect of the three factors in cube form on the three response variables as a 3D surface plot using a trial version of statistical design software Design Expert® [37] are shown in Fig. 2, Fig. 3 and Fig. 4.

3.3.2. Control pectin films made from 3% solution

The thickness, WVTR, WVP, transparency parameter (ΔE) and whiteness index (WI) results of the 2^3 full factorial design of control pectin films made from 3% solution are summarised in Table 2. Contrast coefficients for calculating the effects of factors on the responses were taken from supporting information Table S1. The experiments 3 and 7 were repeated three times and the standard deviation in thickness, WVTR and ΔE were 0.009, 21.28 and 0.38 respectively. The main effects, two- and three-factor interaction effects, mean sum of squares and the percentage contribution of each effect on the three response variables are provided in supplementary material Table S2.

Analysis for less thickness: The factors P, PT and PTH show a negative effect on thickness while T, H, PH and TH show a positive effect. The negative effect of P along with highest contribution (53%), indicates that the high pH is favourable for obtaining thin films. However, the effect of TH (21%) which has a positive effect is also significant on thickness. This indicates that TH should be low and can be achieved by low T or H. The next significant contribution (11%) comes from PT which has a negative effect. If PT has to be high, at a fixed high desirable P, the T should be low and subsequently a low to moderate H to reduce TH effect.

Analysis for low WVTR: The factors T, PT, PH, TH and PTH show negative effects on WVTR, while the factors P and H show positive effects. The three-factor interaction term PTH has highest influence (38%) and has a negative effect on WVTR indicating that PTH should be high. The next highest contributor is H (25%) which has a positive effect. Temperature, T also has 24% contribution and shows a negative effect on WVTR. This means high temperature and low humidity of drying are preferable. In summary, low H, high T and low P are desirable for to achieve films with low WVTR out of 3% solution of control pectin.

Analysis for high transparency: The factors T, H, PT, PH, TH show negative effects on the transparency parameter ΔE while P and PTH show a positive effect. The highest contribution (81%) and a positive effect by pH (P) indicates that, low pH is favourable to get transparent films (low ΔE value). The next contributing factor is PTH (7%) and PTH has a positive effect on ΔE. In order to reduce the PTH, low H and high T are favourable to get transparent films since we already have zeroed in on low pH.

The influence of two-factor interactions at the base level of the third factor and the combined effect of the three factors in cube form on the three response variables as a 3D surface plot are shown in the supplementary material Fig. S1, Fig. S2 and Fig. S3 for brevity.

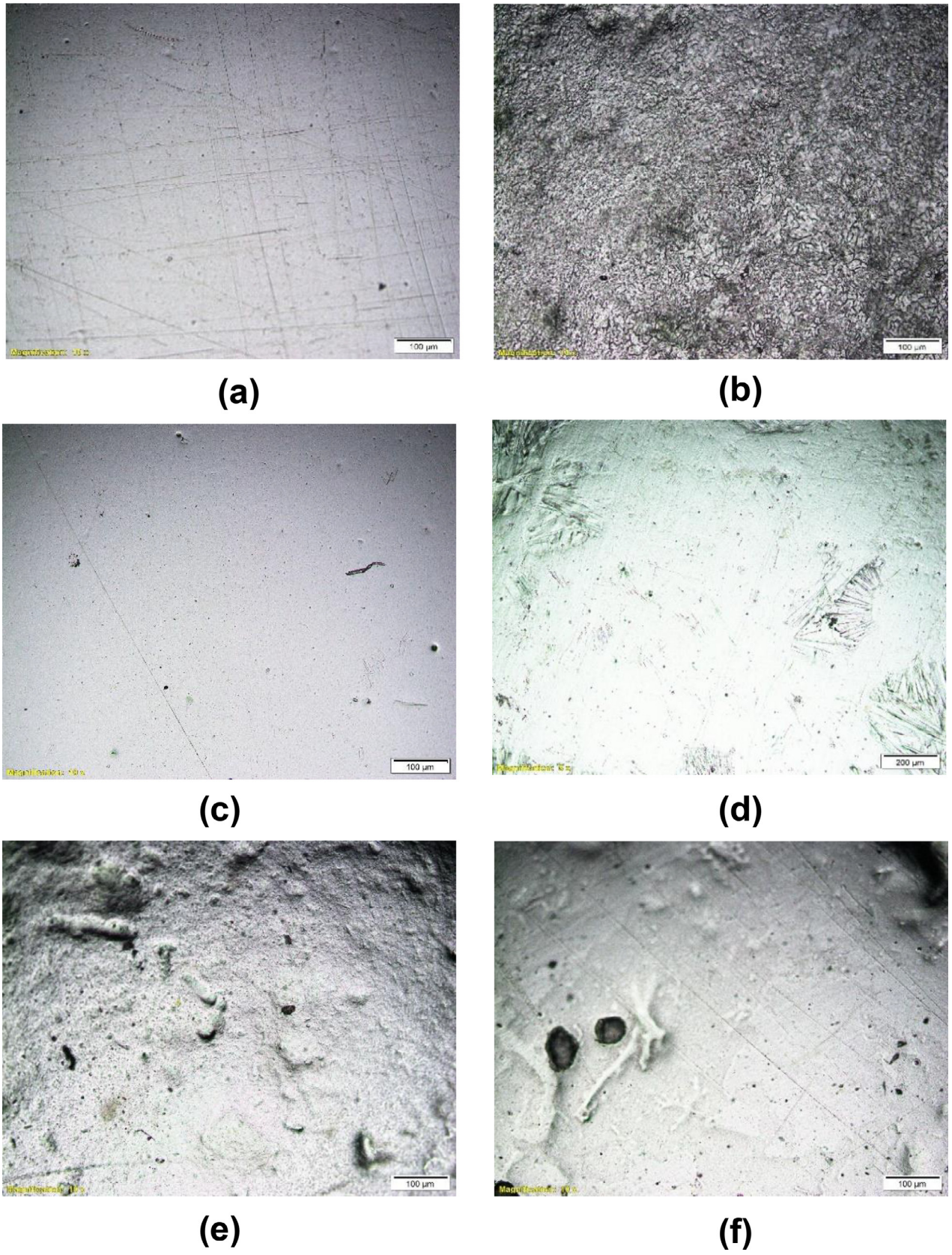


Fig. 1. Surface micrographs of (a) 5% control pectin pH = 3, T = 22°C, H = 40%; (b) 5% control pectin pH = 7, T = 48°C, H = 60%; (c) 5% control pectin pH = 7, T = 22°C, H = 40%; (d) 5% pectin + glycerol pH = 3, T = 22°C, H = 40%; (e) 5% pectin + glycerol pH = 7, T = 48°C, H = 60%; (f) 5% pectin + glycerol pH = 7, T = 22°C, H = 40%.

Table 4
Values of main, two-factor and three-factor interaction effects for control pectin films from 5% w/v solution.

Term	Thickness			WVTR			ΔE		
	Effect	Mean Sum of Squares	% contribution	Effect	Mean Sum of Squares	% contribution	Effect	Mean Sum of Squares	% contribution
P	0.0085	0.0001445	1.68	66.920	8957.24	4.02	9.855	194.242	82.02
T	0.0395	3.12E-03	36.37	−272.868	1.49E + 05	66.85	1.400	3.920	1.65
H	0.005	5.00E-05	0.58	−32.4675	2108.28	0.95	−0.070	0.0098	0.004
P-T	0.013	0.000338	3.94	−14.8775	442.68	0.2	3.870	29.954	12.65
P-H	−0.0025	1.20E-05	0.15	−56.4775	6379.42	2.86	0.440	0.3872	0.16
T-H	−0.0075	1.13E-04	1.31	142.533	40,631	18.24	−1.615	5.216	2.20
P-T-H	−0.049	0.004802	55.97	−87.4775	15304.6	6.87	1.245	3.100	1.31

3.3.3. Pectin + glycerol films made from 5% solution

The thickness, WVTR, WVP, transparency (ΔE) & whiteness index (WI) results of the 2^3 full factorial design of control pectin + glycerol (P:G = 5:1.26 w/w) films made from 5% solution are summarised in Table 3. Contrast coefficients for calculating the effects of factors on the responses are taken from the supporting information Table S1. The experiments 3 and 7 were repeated three times and the standard deviation in thickness, WVTR and ΔE are 0.002, 47.51 and 0.49 respectively. The main effects, two and three-factor interaction effects, Mean sum of squares and the percentage contribution of each effect on the three response variables are shown in Table 5.

Analysis for less thickness: The factors T, PT and PTH show a negative effect on thickness while P, H, PH and TH show a positive effect. The three-factor interaction PTH has highest contribution (35%) and has a negative effect on thickness. Furthermore, the effect of PT (25%) which has a negative effect is also significant for regulating thickness. This indicates that PT should be high and can be achieved by a high T or P. The next significant factors are PH (18%) and TH (19%) which have a positive effect indicating that these values should be low. By comparing all the effects and contributions, it is clear that a high T, low P and low to moderate H are the favourable conditions.

Analysis for low WVTR: The factors P, and T show negative effects on WVTR while the factors H, PT, PH, TH and PTH show a positive effect. Humidity H has highest influence (57%) while has a positive effect on WVTR. This indicates that low humidity of drying is preferred. The three-factor interaction term PTH has 20% influence and has a positive effect on WVTR indicating that PTH should be low. The next highest contributor is T (8%) which has a negative effect indicating that high temperature is favourable.

Analysis for high transparency: The factors T, H and PTH show a negative effect on the transparency ΔE, while the factors P, PT, PH and TH show a positive effect. The highest contribution (37%) and a positive effect by pH (P) indicates that, low pH is favourable to obtain transparent films (low ΔE value). The next contributing factor is PTH (21%), which has negative effects on ΔE. Humidity H has a negative effect and 13% contribution indicating that higher humidity is desirable. PT and PH also have same contribution (12–13%) but having a positive effect on ΔE.

The influence of two-factor interactions at the base level of the third factor and the combined effect of the three-factors in cube form on the three response variables thickness, WVTR and ΔE in the 3D surface plot format are shown in supplementary material Fig. S4, Fig. S5, and Fig. S6 respectively.

3.3.4. Pectin + glycerol films made from 3% solution

The thickness, WVTR, WVP, transparency (ΔE) & whiteness index (WI) results of the 2^3 full factorial design of control pectin + glycerol (P:G = 3:1.26 w/w) films made from 3% solution are summarised in Table 3**.** Contrast coefficients for calculating the effects of factors on the responses are taken from the supporting information Table S1. The experiments 3 and 7 were repeated three times and the standard deviation in thickness, WVTR and ΔE are 0.006, 52.62 and 0.47 respectively. The main effects, two and three-factor interaction effects, mean sum of squares and the percentage contribution of each effect on the three response variables are provided in supplementary material Table S3.

Analysis for less thickness: The factors PT and PTH show negative effects on thickness while P, T, H, PH and TH show a positive effect. The PT interaction term has highest influence (50%) and show negative effects on film thickness indicating that a high PT value is preferred. The three-factor interaction PTH has a 33% contribution and has a negative effect on thickness. Furthermore, the terms showing positive effects such as PH and TH have %-contributions of 4% & 6% respectively. This indicates that PH and TH interaction terms should be low. By comparing all the effects and contributions, it is clear that a high T, low to medium P and low to medium H are the favourable conditions.

Analysis for low WVTR: The factors P, T, PT, PH and TH show negative effects on WVTR while the factors H, and PTH show positive effects. The factor P has highest influence (59%) with a negative effect on WVTR. This indicates that a high pH is preferrable. The two-factor interaction term PT has 28% influence and has negative effects on WVTR indicating that PT should be high. The next highest contributor is T (5%) which has negative effects indicating that high temperature is favourable. In summary, low to medium humidity, high temperature and medium to high pH are desirable to obtain 3% pectin + glycerol films with low WVTR.

Analysis for high transparency: The factors T, H, PT and PH show negative effects on the transparency ΔE, while the factors P, TH and PTH show positive effects on ΔE. The highest contribution (53%) with a positive effect by pH (P) indicates that, low pH is favourable to achieve high transparent films (low ΔE value). The next contributing factor is H (31%) and H has negative effects on ΔE indicating that high humidity of drying is favourable. The TH interaction term has 6% influence with a positive effect indicating that TH should be low. As H should be high, the T can be low.

The influence of two-factor interactions at the base level of the third factor and the combined effect of the three factors in cube form on the three response variables thickness, WVTR and ΔE in the 3D surface plot format are shown in the supplementary material Fig. S7, Fig. S8 and Fig. S9 for brevity.

Detailed assessment of the effects of main factors and their interactions on response variables for the four sets of films (32 films in total) developed, revealed that the three response variables (thickness, WVTR and ΔE) are mainly affected by the pH, temperature and their interaction with humidity. Humidity alone does not seem to have any significant effect. The preferred conditions for developing films with less thickness, less WVTR and low ΔE are, acidic or low pH (3), high temperature (48°C) and low to medium humidity values (40–50%).

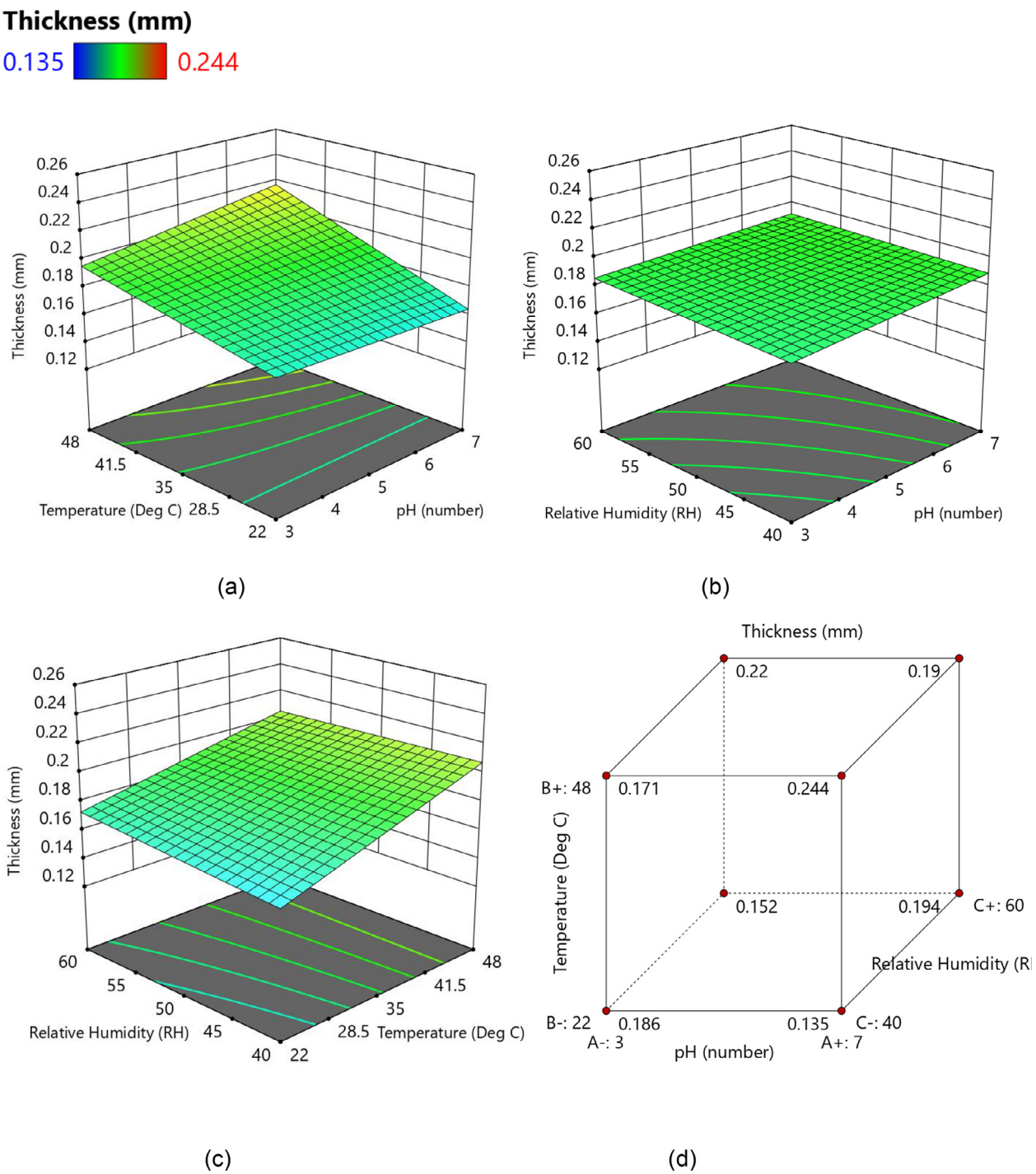


Fig. 2. Control Pectin from 5% solution. Two factor interactions on Thickness (a) effect of interactions of P & T at H = 50%; (b) effect of interactions of P & H at T = 35°C; (c) effect of interaction of H & T at pH = 5; (d) effect of three factors P, T & H.

3.4. Regression models, ANOVA and lack-of-fit analysis

The first-order linear regression models were fitted for different responses (Th, WVTR and ΔE) as a function of independent variables (P, T, H and their interaction terms), which affect the responses significantly. The general model equation along with the model parameters or the coefficients of regression model fitted, analysis of variance (ANOVA) and the details of R^2, model-p value and the *p*-value for individual terms for the three response variables (Th, WVTR and ΔE) for all the four sets of films are reported in Table 6. The R^2 values (Table 6) for all the models are close to 1.0, suggesting good fitness of the models. For instance, comparison of model predicted responses (Th, WVTR and ΔE) with experimental responses for the control pectin films made from 5% solution in Fig. 5 shows a close correspondence, suggesting well representation of responses as a function of different factors. Similar close correspondence was also observed for other three types of films made from different concentrations/composition (supplementary material Fig. S10, Fig. S11 and Fig. S12 for 3% control pectin films, 5% pectin + glycerol films and 3% pectin + glycerol films respectively).

In addition, the check for statistical significance of models and lack-of-fit analysis was performed (Supporting Information

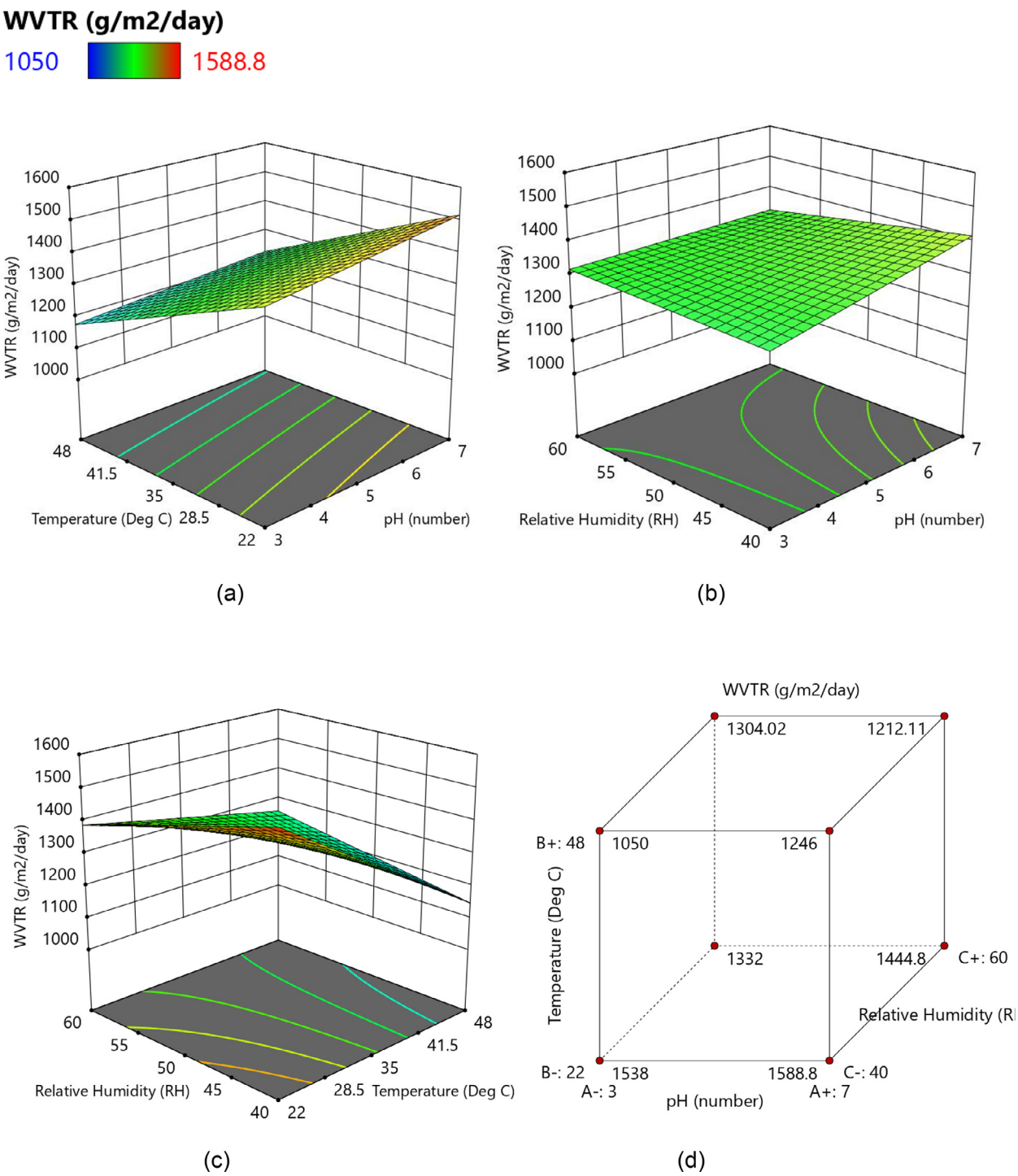


Fig. 3. Control Pectin from 5% solution. Two factor interactions on WVTR (a) effect of interactions of P & T at H = 50% (b) effect of interactions of P & H at T = 35°C; (c) effect of interaction of H & T at pH = 5; (d) effect of three factors P, T & H.

Table S4 and Table S5). Both statistical F-test and p-value showed that the regression models are significant (i.e., regression coefficients are important) and the lack-of-fit is not significant at a 90% confidence level. This shows that the fitted models are adequate approximation to provide good predictions for the desired responses as a function of different factors considered.

4. Conclusions

Four sets of pectin based films were developed with varying concentrations of pectin and glycerol. A 2^3 (two-level three-factor) statistical design of experiments was used to find the optimum process conditions (pH of solution, temperature and relative humidity of drying) for obtaining the targeted film properties, which include less thickness, low WVTR and high transparency. The films made from 5% concentration solution have exhibited improved characteristics compared to those prepared from 3% solution. The extent of improvement depended on the process conditions. Furthermore, glycerol that was used as a plasticizer, increased the moisture absorption of the pectin films due to its hygroscopic nature. Based on the level of statistical significance of all the main and interaction factors effects on the response vari-

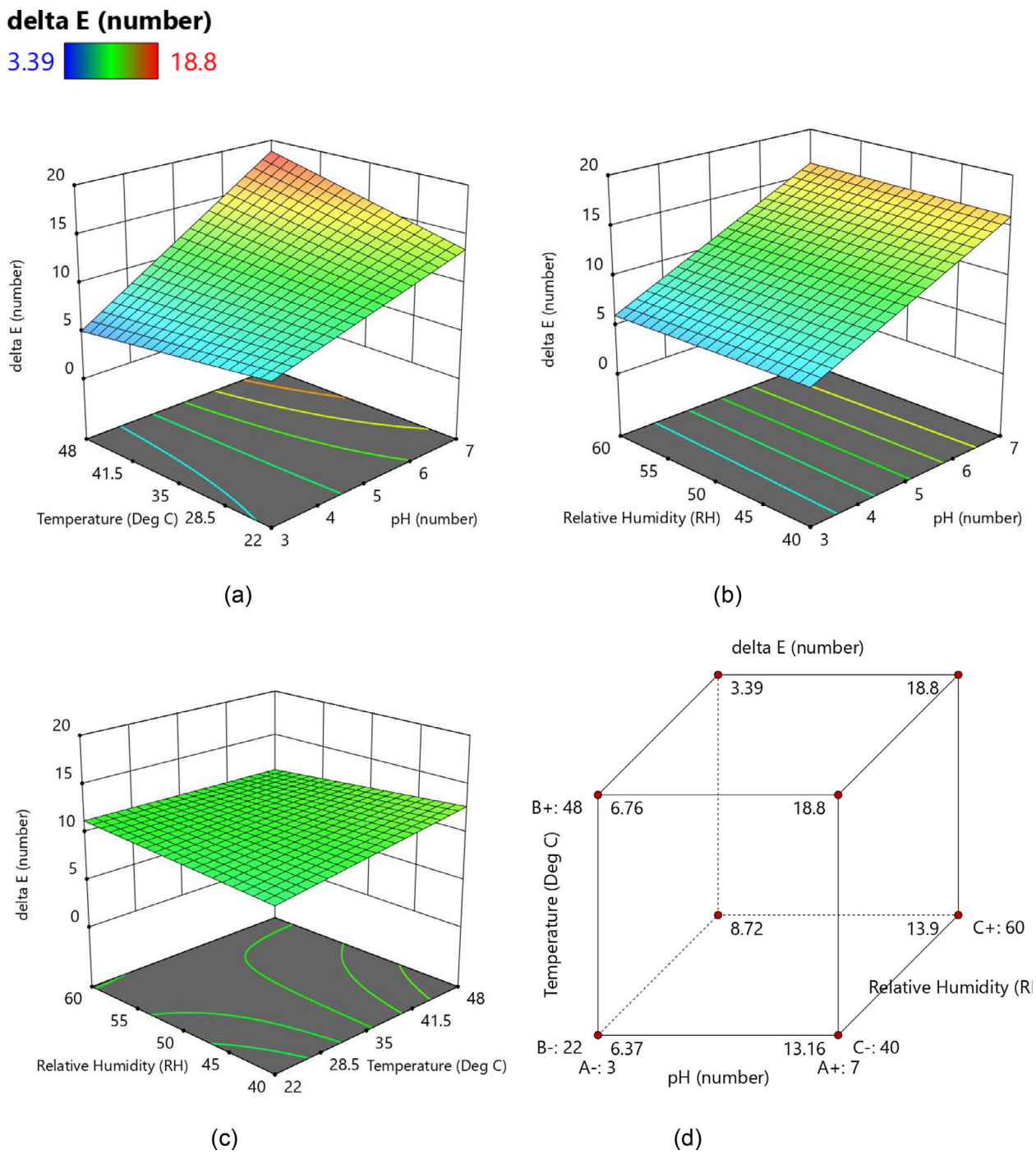


Fig. 4. Control Pectin from 5% solution. Two factor interactions on ΔE (a) effect of interactions of P & T at H = 50%; (b) effect of interactions of P & H at T = 35°C; (c) effect of interaction of H & T at pH = 5; (d) effect of three factors P, T & H.

ables: (a) the factors, pH of the solution and drying temperature, have the most significant effect on the film thickness (b) along with pH and temperature, the effect of relative humidity of drying is also significant on WVTR (c) transparency of the films is affected majorly by pH of the solution. Based on the detailed analysis, the optimal conditions are low pH = 3 and a high T = 48°C and a low to medium relative humidity H of drying (40–50%) for developing films with less thickness and low WVTR and high transparency. Statistical analysis showed that the regression models are significant and the lack-of-fit is not significant at 90% confidence level, suggesting that the fitted models are adequate to describe the effects of different factors on the targeted film properties.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors gratefully acknowledge Northern India Textile Research Association (NITRA), Ghaziabad and Dr. Manish Kumar Chatli, GADVASU, Ludhiana, India for their support with the WVTR analysis.

Table 5
Values of main, two factor and three factor interaction effects for pectin + glycerol films from 5% w/v solution.

Term	Thickness			WVTR			ΔE		
	Standardized Effect	Mean Sum of Squares	% contribution	Standardized Effect	Mean Sum of Squares	% contribution	Standardized Effect	Mean Sum of Squares	% contribution
P	0.01225	0.0003	1.51	−107.57	23142.6098	3.78	6.077	73.872	37.34
T	−0.00775	1.20E-04	0.61	−164.83	54337.8578	8.86	−1.7025	5.80	2.93
H	0.01125	2.50E-04	1.27	418.03	349498.162	57.02	−3.6075	26.08	13.15
P-T	−0.04975	0.0049	24.95	29.23	1708.7858	0.28	3.4275	23.5	11.87
P-H	0.04225	3.60E-03	18	106.37	22629.1538	3.69	3.6425	26.53	13.41
T-H	0.04325	3.70E-03	18.86	130.03	33815.6018	5.52	0.0725	0.0105	0.0053
P-T-H	−0.05875	0.0069	34.8	252.77	127785.346	20.85	−4.5875	42.09	21.27

Table 6
Coefficients of regression model and ANOVA for fitted models of the response variables.

	Control Pectin 5% film			Control Pectin 3% film			Pectin + glycerol 5% film			Pectin + glycerol 3% film		
	Th	WVTR	ΔE	Th	WVTR	ΔE	Th	WVTR	ΔE	Th	WVTR	ΔE
β0	0.1865	1339.416	11.2462	0.1224	1900.84	6.16	0.2126	1866.385	11.069	0.1783	2367.4	8.5225
β1	0.0043[a]	33.4112[a]	4.9367[b]	−0.0464[b]	109.759[b]	2.1525[b]	0.0061[a]		3.0388[b]	0.008[a]	−194.9[b]	3.835[b]
β2	0.0198[b]	−136.484[b]	0.7088[b]		−183.559[b]	−0.5375[b]		−82.415[a]			−57.15[a]	
β3					192.641[b]			209.015[b]	−1.8038[a]		41.9[a]	−2.92[b]
β12	0.0065[a]		1.9437[b]	−0.0206[b]		−0.55[b]	−0.0249[b]		1.7137[a]	−0.031[b]	−134.35[b]	−0.9725[a]
β13		−28.188[a]		0.0159[a]	−78.041[b]		0.0211[b]		1.8212[b]	0.009[a]		−1.1025[a]
β23		71.3163[b]	−0.8163[b]	0.0293[b]			0.0216[b]	65.015[a]		0.0112[a]		1.2975[a]
β123	−0.0245[b]	−43.6888[b]	0.6138[b]	−0.0173[a]	−234.041[b]	0.6525[b]	−0.0294[b]	126.385[b]	−2.2937[b]	−0.0255[b]	39.65[a]	
R^2	0.99	0.96	0.99	0.98	0.99	0.99	0.98	0.92	0.97	0.97	0.97	0.97
Adj R^2	0.95	0.91	0.98	0.94	0.98	0.97	0.93	0.82	0.89	0.9	0.91	0.92
Model-*p*	0.007	0.02	0.003	0.04	0.009	0.003	0.04	0.05	0.06	0.06	0.06	0.05

Regression Model: $Y_{predicted} = \beta_0 + \beta_1 P + \beta_2 T + \beta_3 H + \beta_{12} PT + \beta_{13} PH + \beta_{23} TH + \beta_{123} PTH$.
[a]refers to the terms significant at statistical $p < 0.1$ level.
[b]refers to the terms significant at statistical $p < 0.05$ level.

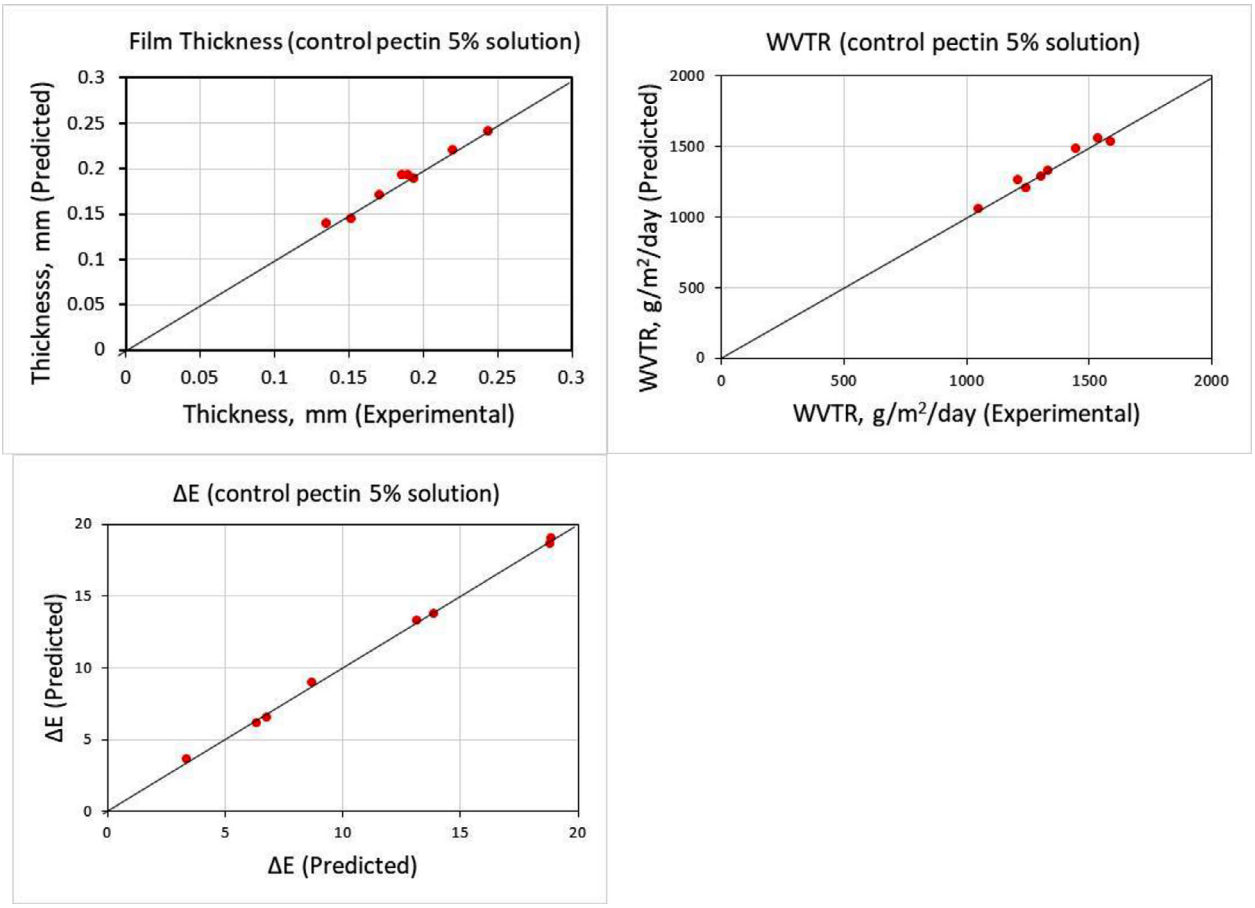


Fig. 5. Comparison of experimental vs regression model for the films developed with control Pectin 5% solution.

Supplementary material

https://doi.org/10.1016/j.ejbt.2021.11.004.

References

[1] Montero Garcia MP, Gomez-Guillen MC, Lopez-Caballero ME, et al. Edible films and coatings: Fundamentals and applications. Taylor Francis: CRC Press; 2017. p. 616. ISBN 9781482218312. https://doi.org/10.1201/9781315373713 PMid: 29106105.

[2] Cerqueira MAPR, Pereira RNC, Ramos PLS, et al. Edible food packaging: Materials and processing technologies. Taylor Francis: CRC Press; 2016. p. 469. ISBN 9781482234169.

[3] Lin D, Zhao Y. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. Compr Rev Food Sci Food Safety 2007;6(3):60–75. https://doi.org/10.1111/crfs.2007.6.issue-310.1111/j.1541-4337.2007.00018.x.

[4] Huber KC, Embuscado ME. Edible films and coatings for food applications. New York, NY: Springer; 2009. p. 416.

[5] Otoni CG, Avena-Bustillos RJ, Azeredo HMC, et al. Recent advances on edible films based on fruits and vegetables—A review. Compr Rev Food Sci Food Safety 2017;16(5):1151–69. https://doi.org/10.1111/1541-4337.12281 PMid: 33371612.

[6] Falguera V, Quintero JP, Jiménez A, et al. Edible films and coatings: Structures, active functions and trends in their use. Trends Food Sci Technol 2011;22 (6):292–303. https://doi.org/10.1016/j.tifs.2011.02.004.

[7] Vaishali, Sharma HP, Samsher, et al. Importance of edible coating on fruits and vegetables: A review. J Pharmacogno Phytochem 2019;8(3):4104–10.

[8] Hoorfar J. Global safety of fresh produce: A handbook of best practice, innovative commercial solutions and case studies. Sawston,UK: Woodhead Publishing Limited; 2014. p. 472. ISBN: 9781782420187.

[9] Sanchez-Ortega I, García-Almendárez BE, Santos-Lopez EM, et al. Antimicrobial edible films and coatings for meat and meat products preservation. Sci World J 2014:248935. https://doi.org/10.1155/2014/248935 PMid: 25050387.

[10] Mellinas C, Ramos M, Jimenez A, et al. Recent Trends in the use of pectin from agro-waste residues as a natural-based biopolymer for food packaging applications. Materials 2020;13(3):673–90. https://doi.org/10.3390/ma13030673 PMid: 32028627.

[11] Venkatachalam K, Lekjing S. A chitosan-based edible film with clove essential oil and nisin for improving the quality and shelf life of pork patties in cold storage. RSC Adv 2020;10:17777–86. https://doi.org/10.1039/D0RA02986F.

[12] Cortes-Rodríguez M, Villegas-Yepez C, Gonzalez JHG, et al. Development and evaluation of edible films based on cassava starch, whey protein, and bees wax. Heliyon 2020;6:E04884. https://doi.org/10.1016/j.heliyon.2020.e04884 PMid: 32984596.

[13] Fitch-Vargas PR, Aguilar-Palazuelos E, Zazueta-Morales JJ, et al. Physicochemical and microstructural characterization of corn starch edible films obtained by a combination of extrusion technology and casting technique. J Food Sci 2016;81(9):E2224–32. https://doi.org/10.1111/1750-3841.13416 PMid: 27550869.

[14] Abhishaben MS, Chandegara VK, Jithender B, et al. Whey protein isolate based biodegradable food packaging film as affected by protein to glycerol ratio, pH and sonication amplitude. Int J Curr Microbio Appl Sci 2019;8(3):895–909. https://doi.org/10.20546/ijcmas.2019.803.107.

[15] Abral H, Basri A, Muhammad F, et al. A simple method for improving the properties of the sago starch films prepared by using ultrasonication treatment. Food Hydrocolloids 2019;93:276–83. https://doi.org/10.1016/j.foodhyd.2019.02.012.

[16] Mohnen D. Pectin structure and biosynthesis. Curr Opin in Plant Biol 2008;11 (3):266–77. https://doi.org/10.1016/j.pbi.2008.03.006 PMid: 18486536.

[17] Zandleven J, Sørensen SO, Harholt J, et al. Xylogalacturonan exists in cell walls from various tissues of *Arabidopsis thaliana*. Phytochem 2007;68(8):1219–26. https://doi.org/10.1016/j.phytochem.2007.01.016 PMid: 17336350.

[18] Grassino AN, Halambek J, Djakovic S, et al. Utilization of tomato peel waste from canning factory as a potential source for pectin production and application as tin corrosion inhibitor. Food Hydrocolloids 2016;52:265–74. https://doi.org/10.1016/j.foodhyd.2015.06.020.

[19] Li DQ, Du GM, Jing WW, et al. Combined effects of independent variables on yield and protein content of pectin extracted from sugar beet pulp by citric acid. Carbohydr Polym 2015;129:108–14. https://doi.org/10.1016/j.carbpol.2015.04.058 PMid: 26050895.

[20] Oliveira TÍS, Rosa MF, Cavalcante FL, et al. Optimization of pectin extraction from banana peels with citric acid by using response surface methodology. Food Chem 2016;198:113–8. https://doi.org/10.1016/j.foodchem.2015.08.080 PMid: 26769512.

[21] Valdes A, Burgos N, Jimenez A, et al. Natural pectin polysaccharides as edible coatings. Coatings 2015;5(4):865–86. https://doi.org/10.3390/coatings5040865.

[22] Batori V, Jabbari M, Akesson D, et al. Production of pectin-cellulose biofilms: A new approach for citrus waste recycling. Inter J Polym Sci 2017;9732329. https://doi.org/10.1155/2017/9732329.

[23] Sartori T, Feltrea G, Sobral PJA, et al. Properties of films produced from blends of pectin and gluten. Food Packag Shelf Life 2018;18:221–9. https://doi.org/10.1016/j.fpsl.2018.11.007.

[24] Chakravarthula SSN, Michela S, Nadia L, et al. Characterization of composite edible films based on pectin/alginate/whey protein concentrate. Materials 2019;12(15):2454–73. https://doi.org/10.3390/ma12152454 PMid: 31374873.

[25] Cabello PSD, Takara EA, Marchese J, et al. Influence of plasticizers in pectin films: Microstructural changes. Mater Chem Phys 2015;162:491–7. https://doi.org/10.1016/j.matchemphys.2015.06.019.

[26] Mendes JF, Norcino LB, Manrich A, et al. Characterization of pectin films integrated with cocoa butter by continuous casting: physical, thermal and barrier properties. J Polym Enviro 2020;28:2905–17. https://doi.org/10.1007/s10924-020-01829-1.

[27] Phuong Ngo TM, Nguyen TH, Dang TMQ, et al. Characteristics and antimicrobial properties of active edible films based on pectin and nanochitosan. Int J Mol Sci 2020;21:2224–40. https://doi.org/10.3390/ijms21062224 PMid:32210135.

[28] Priyadarshi R, Kim SM, Jong-Whan R. Pectin/pullulan blend films for food packaging: Effect of blending ratio. Food Chem 2021;347. https://doi.org/10.1016/j.foodchem.2021.129022 PMid: 33482482.

[29] Younis HGR, Abdellatif HRS, Ye F, et al. Tuning the physicochemical properties of apple pectin films by incorporating chitosan/pectin fiber. Int J Biol Macromol 2020;159:213–21. https://doi.org/10.1016/j.ijbiomac.2020.05.060 PMid: 32416291.

[30] Rojas-Grau MA, Soliva-Fortuny R, Martín-Belloso O. Edible coatings to incorporate active ingredients to fresh-cut fruits: A review. Trends Food Sci Technol 2009;20:438–47. https://doi.org/10.1016/j.tifs.2009.05.002.

[31] Gurram R, Souza Filho PF, Taherzadeh MJ, et al. A solvent-free approach for production of films from pectin and fungal biomass. J Polym Environ 2018;26:4282–92. https://doi.org/10.1007/s10924-018-1300-x.

[32] Smith JM, Van Ness HC, Abbott MM, et al. Introduction to chemical engineering thermodynamics. Mc Graw Hill Education; 2018. ISBN: 978-1259696527.

[33] Montgomery DC, Runger GC. Applied statistics and probability for engineers. 3rd edition. Hoboken: John Wiley & Sons Inc; 2003.

[34] Solak AO, Dyankova SN. Composite films from sodium alginate and high methoxyl pectin - Physicochemical properties and biodegradation in soil. Ecologia Balkanica 2014;6(2):25–34.

[35] Liu L, Kerry JF, Kerry JP. Effect of food ingredients and selected lipids on the physical properties of extruded edible films/casings. Int J Food Sci Technol 2006;41(3):295–302. https://doi.org/10.1111/j.1365-2621.2005.01063.x.

[36] Tapia-Blacido DR, Sobral PJA, Menegalli FC. Optimization of amaranth flour films plasticized with glycerol and sorbitol by multi-response analysis. LWT - Food Sci Technol 2011;44(8):1731–8. https://doi.org/10.1016/j.lwt.2011.04.004.

[37] Design-Expert® Software, Version 13, Stat-Ease Inc., Minneapolis, MN, USA.