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Research article

Optimization of citron peel pectin and glycerol concentration in the production of edible film using response surface methodology

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ABSTRACT

Pectin-based edible film plasticized with glycerol has been developed, and the effect of pectin and glycerol concentration was optimized using response surface methodology for better mechanical properties and transparency. The upper and lower concentration of pectin (3–5 g) and glycerol (15%–25%) concentration ranges were considered in this study based on the preliminary experiment. The responses of the edible film determined were tensile strength, elongation at break and elastic modulus and opacity. The interaction effects of glycerol and pectin concentrations on edible film properties significantly affected the film properties. Tensile strength and opacity were positively affected by pectin concentrations; however, elastic modulus and elongation at break were negatively affected. Glycerol concentration negatively affected the edible film's tensile strength and elastic modulus. The decrease in the opacity of the biofilm was observed as the pectin concentration increased; however, glycerol had not shown a significant influence on opacity. The numerical optimization provided 4 g of pectin, and 20% of glycerol showed a strong and transparent edible film. The TGA curve showed that the maximum weight loss occurred between the temperatures 250–400 °C due to the loss of polysaccharides. From FTIR analysis, observed peaks around 1037 cm^{-1} represented the C–O–C stretching vibrations of the saccharide found in pectin and glycerol.

1. Introduction

Food packaging is an important part of the food industry, and it facilitates the transportation of food products in safer conditions and maintains quality throughout the supply chain [1]. Environmental concerns over the increased production and usage of non-biodegradable packaging materials and problems associated with trash disposal have stoked interest in developing bio-based packaging materials. Hence, substituting non-degradable petrochemical-based packaging films with edible or biodegradable materials is highly required [2]. Various researchers have successfully employed different biodegradable natural polymers, including proteins and polysaccharides, to prepare environmentally friendly edible films and coatings. The edible films containing polysaccharides and proteins can prevent food oxidation, moisture migration from and to the food, and loss of flavor and fragrance.

Additionally, it prevents microbiological contamination. Hence, edible films are now considered the ideal packaging materials for food products. The edible biodegraded films containing carbohydrates have lower moisture barrier properties due to their hydrophilic

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nature [3]. The capability of at least one ingredient to provide the structural matrix with sufficient cohesiveness is necessary to form edible biodegradable films. Pectin is a plant-based polymer with benefits, including being non-toxic, affordable, easily accessible in nature, and biodegradable [4]. Pectin is a known plant cell component found primarily in cell walls and the middle lamella of plant tissues. It is a complex polysaccharide derived from plants extensively employed in various food and pharmaceutical applications [5–7]. Pectin is a galacturonic acid polymer containing an acidic polysaccharide and a methyl ester that also contains an acidic portion. It is characterized as a hydrocolloid with very good thickening and gelling properties and the ability to hold a higher amount of water [8].

Even though pectin is available in many plants, the major sources for its commercial extraction are citrus peel, apple and sugar beet pomace, cocoa husks, mulberry branch bark, peach pomace, sunflower seeds, mango tree bark, watermelon rind, sisal waste, pomegranate peel, and papaya peel [9]. However, other citrus fruits, such as citron fruit and mandarin, are also known as good sources of pectin [5]. Pectin is composed of a backbone of 1–4 galacturonic acid, to which a neutral sugar is linked to form a side chain. Depending on the plant source, some of this galacturonic acid is methyl-esterified at the C-6 carboxyl and sometimes O-acetylated at the O-2 or O-3 positions [10].

The mechanical properties of edible films are influenced by polymer structure, amounts and type of plasticizer, and solvent used. Due to its stability and compatibility with the hydrophilic biopolymer chain, glycerol is the most extensively used plasticizer in edible film production. The modification of electrostatic forces and interactions among the polymer chains results in variations in molecular packing and inter-chain connections in the film matrix; this also alters the film's microstructure and physical attributes [11]. Films developed by solvent casting showed improved mechanical properties and reduced water vapor permeability [12]. These films exhibited high resistance to enzymatic degradation when exposed to simulated intestinal fluid, an important property for colon-targeted delivery systems.

Several studies have reported on the characterization of the pectin-based film incorporating different organic materials [13–15]. However, the optimization of the ingredients was not successfully conducted. The response surface methodology (RSM) is a statistical technique for designing experiments and developing models while evaluating the effects of factors, reducing the number of experimental runs, and identifying possible interactions [16]. However, limited research was reported on developing pectin-based edible biofilm from the citron peel with glycerol as a plasticizer and optimization using response surface methodology.

Considering all the above gaps, this experiment was conducted to develop edible film from citron peel pectin and optimize the pectin and glycerol concentrations for the best quality pectin edible film. The pectin-based edible films were characterized by thermal and functional (FTIR) properties to obtain optimized process conditions.

2. Materials and methods

2.1. Raw materials procurements

The citron peel used in this study was collected from the nearby fruit market to extract pectin. The glycerol (≥99.0% purity) was purchased from a local chemical supplier. Analytical grade chemicals and distilled water have been used in various steps of edible film preparation.

2.2. Microwave-assisted pectin extraction

Microwave extraction of pectin was performed according to the methods described earlier [17]. First, the dried citron peel was measured and placed into a 250 ml beaker, and distilled water was added up to the solid-to-liquid ratio at 1:30 g ml^{-1}, and the pH was adjusted to 1.5. Then, the mixture was placed in the center of the microwave oven over a rotating base and exposed to microwave radiation at 450 W for 2 min. Further, the mixture was filtered, and the remaining impurities were removed using centrifugation at 4000 rpm for 20 min. The supernatant was subjected to precipitation using ethanol (96%) in an equal volume and allowed to stay overnight at 4 °C. Then, the precipitated pectin was isolated by filtration and washed three times with ethanol (96%) to remove the mono and disaccharides [18]. The washed pectin mass was dried at 45 °C for 18 h to obtain dried pectin.

2.3. Experimental design

Response Surface Methodology was used for the design and analysis of this experiment by using the Design Expert Version 12 (Stat-Ease, Minneapolis, USA). A central composite rotatable design of 2 variables with two levels was used to evaluate the response patterns and to determine the optimum combination of variables. The independent variables considered in this experiment were citron peel

Table 1
Independent variable values of the process and their corresponding levels of pectin film development process.

Independent variable	Unit	Level		
		−1	0	+1
Pectin	g	3	4	5
Glycerol	%	15	20	25

pectin and glycerol concentrations (Table 1) based on preliminary research with the best combination of glycerol concentrations. The optimum concentrations of the citron peel pectin and glycerol concentration were considered based on the medium values of the films' three responses (tensile strength, thickness and opacity) were optimized. The experimental results were fitted to a quadratic polynomial model to investigate the interaction between independent variables and to optimize the extraction process considering tensile strength, thickness and opacity of film as responses. The generalized form of the quadratic polynomial model was given in Eq. (1).

$$\gamma = \beta_o + B_1X_1 + B_2X_2 + B_{11}X_1^2 + B_{22}X_2^2 + B_{21}X_1X_2 \quad (1)$$

where: γ = dependent variable examined; β_o = the value of fitted response at the central point of the design (0,0); B_1 and B_2 are the linear regression coefficients; B_{11} and B_{22} are thequadratic regression coefficients, B_{12} is the cross-product regression coefficient; X_1 and X_2 are the independent variables (pectin and glycerol concentration).

2.4. Preparation of edible film

Films were prepared by the solution casting method according to the method described by Yue Chen [19] with slight modifications. Initially, 3–5 g of pectin (according to the experimental run) was dissolved in 100 ml of distilled water. The solution was heated at 70 °C, and 15%–25% (according to the experimental run) of glycerol was added after 20 min and heated for an additional 10 min. The solution was cooled to 25 °C and poured into a cleaned Petri plate (Diameter: 15 cm). The Petri plates were dried for 48 h at 40 °C in a vacuum drying oven. The dried films were peeled-off and packed in a polyethylene plastic bag for further analysis.

2.5. Determination of mechanical properties of pectin films

The tensile mechanical properties were determined with a texture analyzer (XLW (EC), CHINA) following previous reported ASTM procedure [19]. The mechanical properties of the films, including tensile strength (MPa), elongation at break (%) and elastic modulus, were measured at 23 °C (the laboratory temperature), 50% RH (Relative Humidity) and 50 mm min^{-1} speed. Film samples used in tests were cut with sharp scissors into dimensions of 10 mm length and 1 mm width. Tensile Strength (TS), elongation at break (EAB), and elastic modulus (EM) were determined in triplicate.

2.6. Determination of the opacity of pectin films

The opacity of all samples was recorded according to previous study method [20]. The films were cut into rectangular specimens and placed directly in the spectrophotometer test cell (cuvette) using air as a reference. The light transmittance was measured by scanning the samples at wavelengths ranging from 200 to 800 nm using a UV–Visible spectrophotometer (UV–1800 Japan). The opacity of the films was recorded at 600 nm and was calculated as Eq. (2)

$$\text{Opacity} = \frac{A_{600}}{X} \quad (2)$$

where: A_{600} = Absorbance at 600 nm, X = Thickness (mm). The measurement was repeated three times for each type of film, and the reported result is the mean of these measures.

2.7. Characterization of optimized pectin film

2.7.1. Thermogravimetric analysis

A thermogravimetric analyzer (TGA HTC-1 Instruments, USA) with a regular flow of nitrogen gas provided at a rate of 20 ml min^{-1} was employed to determine the thermal stability of 10 g of prepared edible pectin film in a temperature between 25 °C and 700 °C at a heating rate of 10 °C.min^{-1}. The weight loss rates due to the thermal decomposition within the heating range were calculated by comparing the original weight with the weight loss data obtained [21].

2.7.2. Fourier transform infrared spectroscopy analysis

The Fourier Transform Infrared (FT-IR) (Nicolet, ISO50, USA) spectra of film from the optimal composition were produced. Spectra were recorded in transmission mode within the wavelength range of 4000–500 cm^{-1} with 32 scans per spectrum at a resolution of 16 cm^{-1}. Data from FTIR measurements were achieved over the range and analyzed with Origin Pro software [21].

2.7.3. Statistical Analysis and model fitting

By applying multiple regression analysis on the experimental data, a polynomial model was generated by Design Expert 12 software to predict tensile strength, elastic modulus, elongation at break, and opacity as a function of process variables. The model equation is expressed in X1 and X2, which represent pectin and glycerol concentrations, respectively. The statistical significance of the proposed quadratic model for responses (tensile strength, elastic modulus, and opacity) was evaluated by ANOVA. The numerical optimization was considered to determine the optimum concentrations of the pectin and glycerol concentration for the edible film with optimum properties [22].

3. Result and discussion

3.1. The interaction effect of parameters on mechanical properties

3.1.1. The effect of parameters on tensile strength

Tensile strength is very important parameater which represents the strength of the film withstand for the force. The pectin edifilm from this study is showed the tensile strength of 4.00–7.001 Mpa. In this study, the tensile strength of pectin-based films showed a statistically significant ($P < 0.05$) effect in the linear, quadratic and interactive models of pectin and glycerol concentration (Table 3). Pectin films prepared in this study exhibited increased tensile strength by increasing pectin concentration. The effect of studied parameters on tensile strength is shown in Fig. 1a. The result indicated that as the concentration of pectin increased, the tensile strength of the prepared edible film also increased. This increase in tensile strength is attributed to forming of a polysaccharide network during film drying [23]. The proximity of pectin chains is favored by higher pectin content, which leads to the formation of a denser matrix. Increasing the glycerol concentration in the film-forming solution produced weaker and more deformable films. Similerly, additives in the pectin film formation solution also decreased the tensile strength of the pectin films. For instance, addition of essential oils reduced the tensile strength of the pectin edible films [24]. The quantities and sources of the pectin, additives are directly influencing the tesnile strength of the films (Table 4).

As glycerol concentration increased from 15% to 25% in the film forming solution, the tensile strength of the film decreased from 7.001 to 4 MPa (Table 2). This decrease in the tensile strength is attributed to the insertion of glycerol through the polymer chains and increasing the free space and mobility of the polymers, hence observing the decrease of the mechanical strength of pectin films [25]. It is a common observation that, addition of lower molecular weight plasticizers (like glycerol, sorbitol, polyethylene glycerol) in the pectin film formation solution increases the chain segmental mobility. This leads to the reduction of the cohesion and firmness of the pectin film, while improving its extensibility and flexibility (Biliaderis et al., 1999; Espitia et al., 2014). Incorporation of additives in the pectin film formation solution also brings the variability in the barrier properties to the water vaper and aeroma compounds and gases.

This result of the present study is in consistent with previous studies where the increase in glycerol concentration decreased the

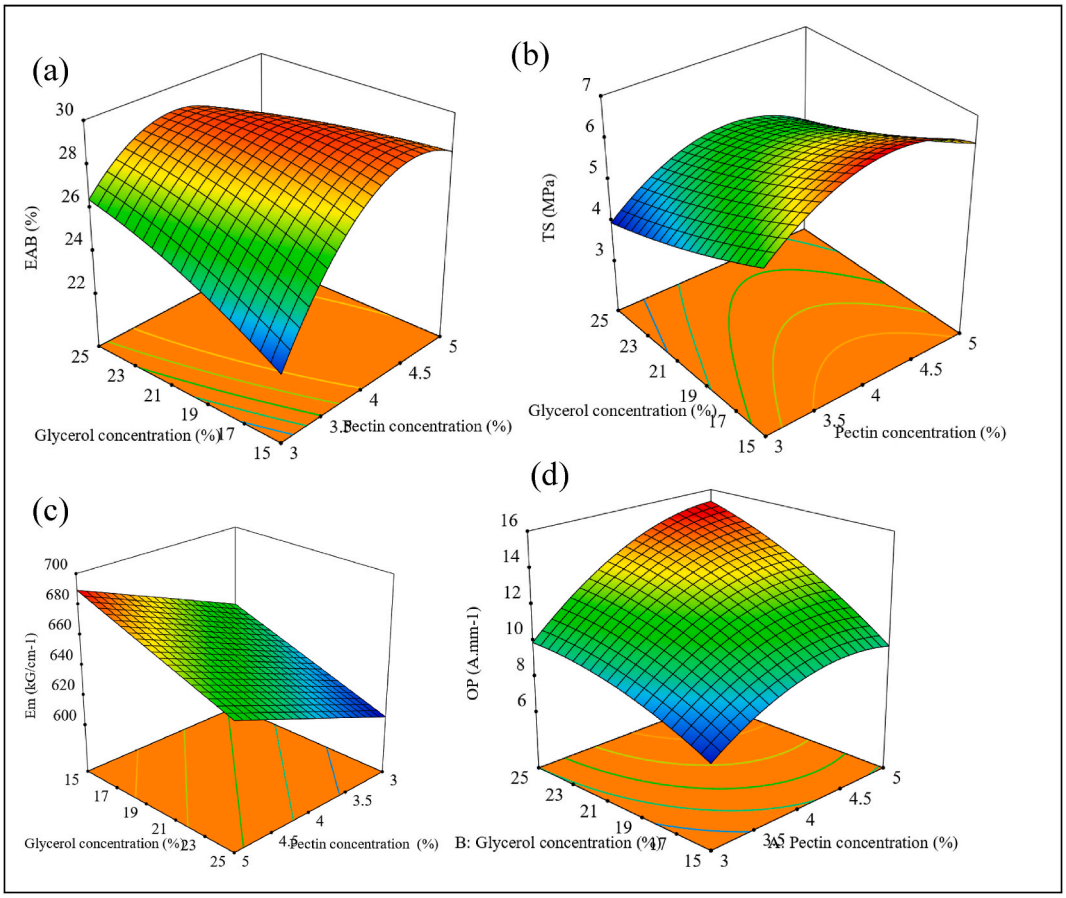

Fig. 1. Response surface plot for the effect of pectin and glycerol concentration on (a) Elongation at break, (b) Tensile strength, (c) Elastic modulus, and (d) Opacity.

Table 2
The measured mechanical properties of the pectin-based film prepared from different concentrations of pectin and glycerol.

S. No	X1	X2	TS (MPa)	EM (kg cm^{-1})	EAB (%)	OP (A mm^{-1})
1	3	15	5.59	640.35	22.21	7.23
2	5	15	6.42	690.12	28.23	9.32
3	3	25	4.00	607.013	26.5	10.25
4	5	25	4.81	645.23	26.2	15.21
5	3	20	4.75	625.32	25.23	8.96
6	5	20	5.21	670.36	27.23	13.68
7	4	15	7.001	670.12	28.5	10.23
8	4	25	5.08	630.25	28.5	13.65
9	4	20	5.90	643.63	29	12.23
10	4	20	6.12	645.56	29.5	12.9
11	4	20	6.01	650.29	28.99	12.36
12	4	20	6.02	650.0	29.11	12.21
13	4	20	6.03	651.01	28.26	12.45

X_1 = Pectin concentration (g); X_2 = Glycerol concentration (%).
TS = Tensile strength; EM = Elastic modulus; EAB = Elongation at break; OP = Opacity.

Table 3
P- values obtained by the Analyze of Variance (ANOVA) of the film properties.

Model	Tensile strength (MPa)	Elastic modulus (kg cm^{-1})	Opacity (A mm^{-1})	Elongation at break (%)
X_1	0.0029	<0.0001	0.0002	0.0006
X_2	<0.0001	<0.0001	0.0001	0.0782
X_1*X_2	0.9521	0.1142	0.0294	0.0006
X_1^2	0.0002	0.4031	0.0070	0.0001
X_2^2	0.2397	0.7607	0.0676	0.0909

X1 = Pectin concentration (g); X2 = Glycerol concentration (%).

edible films' tensile strength. Nisar [26] reported the tensile strength of pectin film varied from 14.78 MPa to 3.78 MPa as pectin concentration increased. Ahmadi [27] reported that psyllium hydrocolloid films' strength decreased as the glycerol concentration increased. Khairunnisa [28] reported that as the glycerol concentration increased from 3% to 90%, the tensile strength of the alginate film decreased from 23.92 MPa to 4.73 MPa. As summarized in Table 4, previous studies also reported that addition of glycerol in film forming solution influenceces the tensile strength of the pectin films.

3.1.2. The effect of parameters on elastic modulus

Elastic modulus denotes the stiffness of a film. The effect of pectin and glycerol concentration on elastic modulus is shown in Fig. 1b. The elastic modulus of pectin films produced in this study are ranged from 607.01 to 690.12 kg cm^{-1} when pectin and glycerol concentrations were varied (Table 2). The effect of the pectin and glycerol concentration on the elastic modulus of the pectin film significantly ($P < 0.05$) affected all the tested models (Table 3).

The elastic modulus of the prepared film decreased as pectin concentration increased in the film-forming solution. As the pectin concentration increased in the pectin film formulation, the brittleness of the films became stiffer. Previous authors reported similar observations on the effects of pectin on the mechanical properties of polysaccharide-based gums [29]. This trend in the elastic modulus of the pectin film may be attributed to the strong hydrogen bonds between starch and intermolecular hydrogen bonds dominated by glycerol and pectin [14].

An increase in the glycerol concentration in the film-forming solution leads to a decrease in the elastic modulus of the pectin films. This decreasing trend in the elastic modulus can be explained as microstructural changes due to the increased amorphous character with an increasing glycerol concentration. However, insignificant improvement in elastic modulus was observed to reduce glycerol concentration and produce a denser and more brittle pectin film. As reported in previous literature, plasticizers are intended to decrease the intermolecular forces along polymer chains, imparting increased film flexibility while decreasing the barrier properties of films [30]. The same is reported in different research findings, as Elina and Nora (2019) reported decreasing in the elastic modulus value as isomalt concentration increased as plasticizers in pectin film [31]. However, the elastic modulus of the pectin film produced from the Gamma-aminobutyric acid (5–15%) lower than the present study [32]. The summarized data presented in Table 4 showed that, the addition of the additives and plasticizers influence the elastic modulus value of the prepared pectin films.

3.1.3. The effect of parameters on elongation at break of pectin film

Elongation at break is a critical attribute of food packaging films its measures its plasticity, indicates a film's capacity to resist shape changes without cracking [33]. Usually films with high tensile strength show low elongation at break [34]. The elongation at break value of the pectin based film prepared in this study ranged 22.21–29.5%.

Fig. 1c shows the response surface graph predicting the effects of pectin and glycerol concentrations on elongation at break. It is observed from this study that increased glycerol concentrations in film-forming solution could result in higher elongation at break

(22.50–29.11%) values for the pectin-based edible film (Table 2). The ANOVA analysis showed that elongation at break is significantly ($P < 0.05$) affected by the pectin and glycerol concentrations in all the studied terms (Table 3). The elongation at break results of the pectin film agree with the finding reported by a previous study [35] that the effect of glycerol on the mechanical properties of pectin film. Sartori [36] suggested the increase in elongation at break is due to the increased polymer chain mobility in the presence of glycerol as pectin concentration increased the elongation of films enhanced and decreased at a high level of pectin. Hydrophilic regions aggregate in the dehydrated condition and the absence of glycerol, generating hydrogen bonds that lower the amount of bound water. These hydrophilic sites become available with the addition of glycerol, and a greater number of water molecules connect to these areas, raising the network's water content. Several result were reported similar result on the effect of pectin concentration on elongation at break of film (Table 4).

3.1.4. The effect of parameters on the opacity of pectin film

Opacity indicates the transparency of a film; the higher opacity value of a film indicates lower transparency [37]. The transparency of the film is important if the film is used as a superficial food coating or wrapping purpose. To modify the apperence properties adjustment of color shade and the opacity is very important [38]. In this study, the opacity of the pectin film ranged from 6.03 to 14.09 (A mm^{-1}) (Table 3). This study's opacity trends of the pectin film showed that the most transparent film was formed at the lowest pectin concentration in the film-forming solutions. The pectin caused this effect by blocking light from passing through the film, increasing the opacity [39]. Fig. 1d shows that the transparency of the film decreases as the concentration of pectin increases while the opacity of the film increases.

Similarly, a higher concentration of glycerol reduced the transparency of the film. A similar trend was observed in a previous study [40], where the concentration of glycerol reduced the transparency of a salep-based edible film. Increasing the solid content significantly increases film opacity, and an increase in opacity may be related to the polymer chain intervals and thickness. Increasing in dry material increases the number of established bonds and thickness of the pectin film; this decreases the light penetration and reduces the transparency [31]. The results showed that the pectin and glycerol content significantly affected the film's opacity, as shown in Table 3. The most transparent and effective film was made with low pectin and a medium glycerol average, which did not impact the film's other mechanical properties [36]. It is clearly evident from the summarized Table 4, that the variation in the pectin film forming solution varied significantly influenced the transparency of the film.

Usually, the pectin films are transparent, as the additives are added the transparency of the films reported to be decreased. In addition, opacity of the pectin film also influences the thickness. The pectin films with the lowest transparency are advantage in packaging of photo oxidative sensitive food products [41]. The optical properties like opacity is an essential sensory parameter for the edible coatings accepted by the consumers. The edible coatings are expected to be colorless and similar to the regular (polymeric) packaging materials or close to the food color onto which are coated [42].

The regression models of the Tensile strength, Elastic Modulus, Elongation at Break and Opacity of the pectin-based edible film in the present is presented in Table 5.

Table 4
The summery table on properties (Tensile strength; Elastic modulus; Elongation at break; Opacity*)* of the pectin based edible film reported by different authors.

S. No	Composition	Properties of the pectin based edible film				Reference
		TS (MPa)	EM (kg cm^{-1})	EAB (%)	OP (A mm^{-1})	
1	Pectin, Glycerol	4.00 to 7.001	607.013–670.36	22.21–29.5	7.23–15.21	Present study
2	Gamma-aminobutyric acid, Pectin	1.43 to 6.41	5.41 to 103.36	8.78 to 32.15	NR	[43]
3	Glycerol, Poly Ethylene Glycol, Pectin	4.48 to 69.33	6.1 to 3270	NR	NR	[14]
4	Pectin, Alginate	22.5 to 42.3	NR	5.9 to 14.9	NR	[4]
5	Pectin, Glycerol	8.9 to 24.3	NR	4.9 to 12.4	NR	[44]
6	Pectin, Zinc Oxide	14.3 to 22.7	NR	5.6 to 8.3	NR	[45]
7	Citrus pectin, high amylose starch, Glycerin	34 to 270	170–760	1.2 to >13	NR	[46]
8	Pectin, gelatin	100.0 to 140.0	NR	17.8 to 21.88	NR	[47]
9	Pectin, calcium ions, putrescine and spermidine	10 to 40	NR	2 to 25	NR	[48]
10	Pectin, chitosan glycerin	7.9 to 12.1	NR	109 to 263.2	NR	[49]
11	Pectin, pomegranate juice and citric acid	1.82 to 10.64	7.58 to 92.70	2.00–20.15	NR	[50]
12	Pectin films, Copaiba oil	12.4 to 41.8	853 to 1589	1.7–2.4	NR	[51]
13	Pectin, sage leaf extract	13.90 to 23.31	NR	15.82–18.82	NR	[52]
14	Gelatin and pectin	8.22 to 21.59	0.28 to 0.5	151.57 to 159.1	NR	[53]
15	Watermelon rind pectin, kiwifruit peel extract	21.65 to 42.30	NR	10.77 to 20.32	NR	[54]
16	Pectin, Alginate, Whey Protein Concentrate	11 to 62	288 to 1467	16 to 40	2.9 to 6.0	[42]
17	Alginate and pectin	20 to 60	NR	6 to 16	0.5 to 0.83	[55]
18	Fish gelatin, Orange peel pectin	6.23 to 10.25		6.23 to 14.36	1.09 to 1.57	[56]
19	Citrus pectin, clove bud essential oil	14.78 to 33.78	2.32 to 2.87	6.37 to 11.75	0.75 to 3.15	[26]
20	Apple pectin, chitosan	1.22 to 6.49	NR	22.09 to 43.77	1.64 to 10.82	[57]

Where: TS = Tensile strength; EM = Elastic modulus; EAB = Elongation at break*;* OP = Opacity, NR = Not reported.

Table 5
Regression model of the studied parameters of the pectin based edible film.

S. No	Response	Regression model	R^2
1	Tensile strength	$5.96 + 0.35x_1 - 0.85x_2 - 0.005x_1x_2 - 0.92x_1^2 + 0.132x_2^2$	0.983
2	Elastic modulus	$647.73 + 22.17x_1 - 19.68x_2$	0.981
3	Elongation at break	$29.11 + 1.28X_1 + 0.37X_2 - 1.58X_1X_2 - 2.81X_1^2 - 0.4X_2^2$	0.985
4	Opacity	$12.55 + 1.96X_1 + 2.05X_2 + 0.17X_1X_2 - 1.31X_1^2 - 0.69X_2^2$	0.980

X_1 = Pectin concentration (g), X_2 = Glycerol concentration (%).

3.2. Optimum conditions and model validation

Optimization of pectin film formulation was performed to achieve the best properties of pectin-based edible films formulated with glycerol suitable for food packaging applications. Numerical optimization through the desirability function approach was applied to estimate the composition of a film with optimal properties. The desirability function should be close to 1. The tensile strength of the pectin-based edible film was considered maximum in optimizing the edible film as this property is very important to maintain integrity during the food handling. At the same time, the strength of the pectin film is used to decide the desirability of elastic modulus in the range. The opacity of pectin film should be minimized due to the more transparent film being selected for packaging purposes. Based on the effect of independent variables (pectin and glycerol) on the responses (Tensile strength, Elastic modulus, Elongation at break, and Opacity) of film, the optimal conditions for the formulation of this film were obtained as 4.5 g of pectin and 20.12% of glycerol, with a desirability value of 0.967. The resulting film with the optimum concentration gives the pectin edible film with 5.84 MPa, 650.41 kg cm^{-1}, 22.21–29.5% and 10.91 A mm^{-1} for tensile strength, elastic modulus, elongation at break and opacity of film, respectively (Table 6).

3.3. Model validation

The validation experiment was repeated three times, the deviation values were less than 5% for all parameters, and the model that RSM obtained can be accepted and considered valid. The results show that this formulation can be applied to prepare the pectin film with superior properties suitable for food packaging.

3.4. Thermogravimetric analysis of optimized film

Thermogravimetric analysis is essential for determining materials' thermal properties, thermal degradation, and weight loss at various temperatures. The derivative thermogravimetry (DTG) thermogram represents the derivative of the sample's weight with respect to temperature change and provides qualitative and quantitative information about the sample. The DTA curve is the first derivation of the thermogravimetry (TG) curve, illustrating the degradation velocity [58].

The TG curve and derivative DTG curves of the optimal pectin film are shown in Fig. 2. The TGA of the best pectin films could be divided into three major groups [58]. The first region (25–150 °C) corresponds to moisture vaporization. The second region (250–400 °C) revealed a high amount of water loss. This water loss is due to polysaccharide decomposition, extensive thermal decomposition followed by decarboxylation of the carbon ring, the evolution of numerous gaseous products, and the formation of solid char [59]. The third region, between 400 and 800 °C, represents weight loss caused by the formation of partially destroyed solid char stacks with polycyclic aromatic structures. Weight loss after 800 °C is caused by sample decomposition and leads to the release of various gases. The Differential Thermal Analysis (DTA) curve of optimal pectin film in Fig. 2 also shows that thermal degradation occurred at 400 °C, and the second peak was obtained at 600 °C, indicating polymer and plasticizer degradation. This result agreed with the previous study reported by Kaya [60]. Kaya reported the thermal degradation of chitosan film as 29.19%.

Table 6
Predicted and experimental yield of pectin at optimum conditions of the film process.

Film composition	Film Properties								
Pec. (g)	Gly. (%)	TS (MPa)		EM (kg cm^{-1})		EAB (%)		OP (A mm^{-1})	
		Pred.	Obt.	Pred.	Obt.	Pred.	Obt.	Pred.	Obt.
4	20.12	5.844 ± 0.15	5.95 ± 0.1	654.82 ± 3.20	654.82 ± 2.23	29.26 ± 0.021	29.69 ± 0.01	13.39 ± 0.23	12.84 ± 0.32

All the values are Mean ± SD of three observations.
TS = Tensile Strength; EM = Elastic Modulus; EAB = Elongation at Break, OP= Opacity, Pec. = Pectin Concentration, Gly = Glycerol Concentration, Pred. = Predicted, Obt. = Obtained Value.

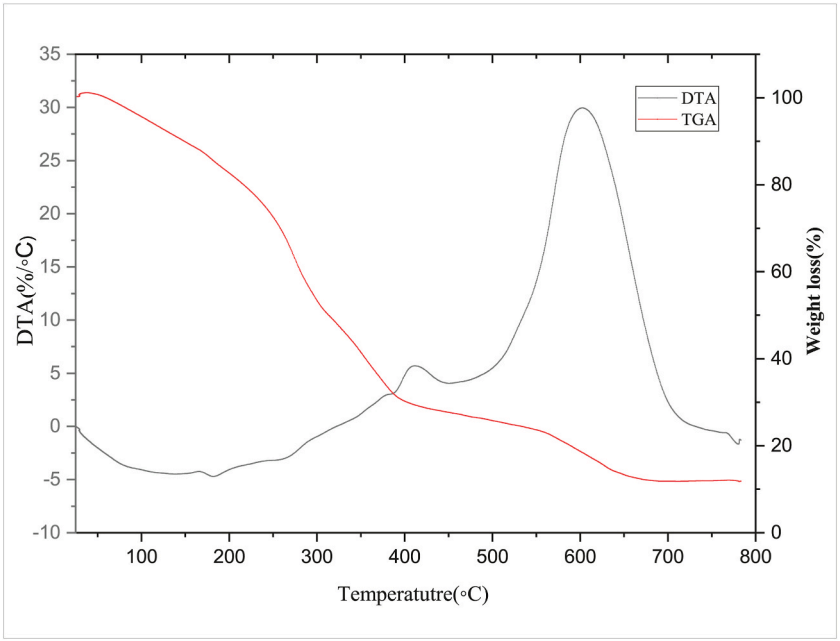

Fig. 2. TGA of pectin-based edible biofilm produced from optimized concentrations of pectin and glycerol.

3.5. Fourier transform infrared (FTIR) analysis of the optimized film

An FTIR spectrum was carried out to study the interactions between the functional groups of the pectin film. The vibration frequency shiftiness of the groups involved in hydrogen bonding due to changes in local electron density can indicate the chain interactions between two polymers. Fig. 3 shows the pectin film's distinctive peaks in the range of 4000–500 cm^{-1}. The broadband around 3291 cm^{-1} is possibly due to the stretching vibrations of intermolecular interactions through the O–H bonds among pectin monomers. The bands at around 2931 cm^{-1} were attributed to the C–H stretching vibrations of methylene groups, the methyl group of pectin polymer chains and the methyl group of the methyl ester [61–63]. The bands at 1715 and 1632 cm^{-1} are assigned to ester stretching vibrations of the CO–CH_3 group and asymmetric stretching vibrations of carboxylate anion –COO^-, respectively, the similar observations are reported in previous findings [14,58]. The bands observed at around 1037 cm^{-1} were assigned to C–O–C stretching vibrations of the saccharide structure. Similar spectral features were observed in pectin and silver nanoparticle composite films studied by previous findings [64]. The spectral differences observed between this film and the literature results could be due to the variations in the film material used and the amount of glycerol used in film-forming solutions.

4. Conclusions

This study used the central composite design to optimize pectin and glycerol amounts for preparing the pectin-based edible film by the solution casting method. In the study, pectin and glycerol concentrations significantly affected the prepared film's tensile strength, elastic modulus, elongation at break, and opacity. As the pectin concentration raised, the tensile strength and elongation at the break of the pectin-based edible film increased. As the glycerol concentration increased reduction in the tensile strength of the prepared pectin-based film was observed. The film's opacity was reduced at higher concentrations of glycerol and negatively affected by pectin concentration. The film developed at optimum pectin and glycerol (4 g and 20.12%) concentrations exhibited strong (high tensile strength) and transparent edible film, which is acceptable. The optimum film showed good thermal properties. Therefore, the film prepared from pectin with glycerol as the plasticizer agent should be considered for the preparation and can be used as good quality food packaging materials. Further study is recommended to develop a pectin-based edible film with different reinforcement to increase the film's mechanical properties. Also, a study on the pectin-based edible films in controlling the bacterial and fungal growth in a part of the packed food preservation should be carried. Further, this optimization study is the concentrations of the pectin and glycerol only, however, there are many other additives influencing the properties of the films. Hence, further research also should carry on optimization of pectin films with different additives.

Author contribution statement

Worku Abera Asfaw: Conceived and designed the experiment; Performed the experiments; Wrote the paper.
Kenenisa Dekeba Tafa: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Neela Satheesh: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

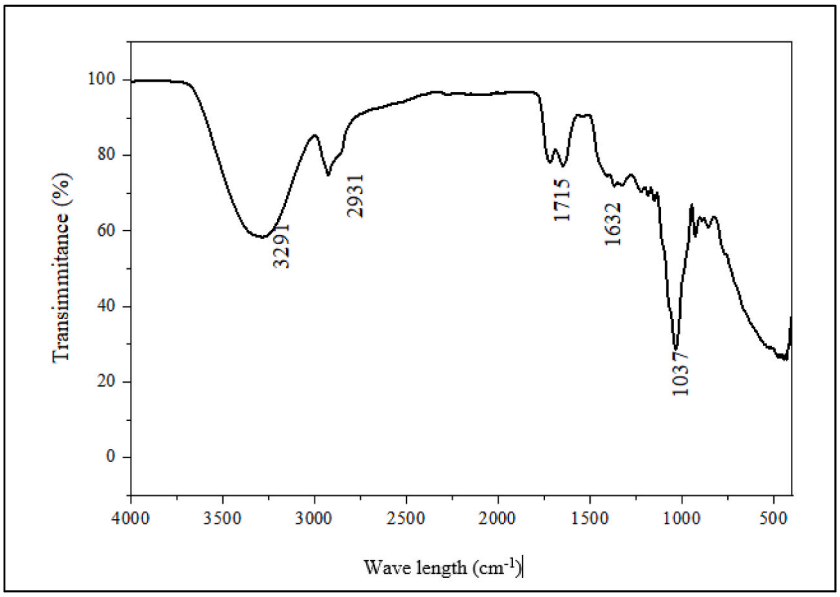

Fig. 3. FTIR of pectin-based edible biofilm produced from optimized concentrations of pectin and glycerol.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

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