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Research article

Mechanical properties of tef starch based edible films: Development and process optimization

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ABSTRACT

The non-biodegradable synthetic plastic is one of the greatest challenges facing the food packaging business since it seriously harms the environment. To solve this problem, non-biodegradable plastic may be disposed of more affordably and with less harm on the environment by using edible starch-based biodegradable film. Therefore, the present study was focused on the development and optimization of tef starch based edible films based on mechanical properties. In this study response surface methodology was employed by considering 3–5g of tef starch, 0.3–0.5% of agar and 0.3–0.5% of glycerol. The prepared film showed the tensile strength of 17.97–24.25 Mpa, elongation break of 1.21–2.03%, elastic modulus of 17.58–108.69 MPa, puncture force of 2.55–15.02 N, puncture formation of 9.59–14.95 mm. The findings showed that as glycerol concentrations in the film-forming solution increased, the prepared tef starch edible films' tensile strength, elastic modulus, and puncture force declined while their elongation at break and puncture deformation increased. Tef starch edible films' mechanical characteristics, including as tensile strength, elastic modulus, and puncture force, were increased by the increase of agar concentration. The optimized (from 5 gm tef starch, 0.4 g agar and 0.3% glycerol) tef starch edible film exhibited higher tensile strength, elastic modulus, and puncture force while lower elongation at break and puncture deformation. The composite edible film based tef starch with agar exhibited good mechanical properties and can be suggested for application in food industry as food packaging.

1. Introduction

According to Plastic Europe (2020), global production of packaging materials has been increased to 368 million tonnes in 2019 and 40.5% of the produced plastics were used for food packaging applications, but less than 1% of packaging materials are biodegredable [1]. Thus, a huge amount of non-biodegradable plastic has been discarded into the environment, which causes the serious environmental pollution. In order to reduce the use of synthetic or non-biodegradable plastic and to preserve environmental health, many researchers are now very interested in producing biodegradable edible films [2,3]. Biodegradable films have been studied and applied as potential alternatives for conventional plastics in food packaging [4,5]. Studies on various types of non-conventional starches have been conducted in response to the growing need for decomposable polymer films for use in food packaging [6]. According to Tye et al.,

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biodegradable edible films are mainly prepared from natural biopolymers, such as carbohydrates, proteins, and lipids [7]. Due to their biodegradability and lack of toxicity, biopolymers including chitosan, carboxymethyl cellulose, starch, and cellophane could be employed to address environmental problems [8].

Starch is most promising polysaccharides for the creation of decomposable edible films since it is a future-proof, abundant, sustainable, and affordable commercial biopolymer [9–11,]. Starch-based biodegradable edible films are now employed in assortment of food packaging and they are environmentally beneficial alternative to synthetic plastics or non-biodegradable films due to their availability and cheap production costs [2,12]. Therefore, starch is considered and appreciated as novel packaging materials for developing starch edible films and can be a prodigious alternate for non-degradable conservative plastic films in different food and nonfood applications [2]. Moreover, starch based biodegradable edible films possess some positive aspects like odorless, tasteless, colorless properties with outstanding mechanical properties [13]. Different investigators are conducted on the preparation and characterization of the edible starch films by cassava, corn, pearl millet, wheat, rice, quinoa, sweet potato, and pea [3,14,15]. However, there is a lack on detailed scientific reports and studies concerning with process optimization and development of tef starch based biodegradable edible films.

Tef is popular and indigenous raw materials that used for preparation of traditional fermented food known as "*Injera*", a staple food in Ethiopia. It contains magnificent nutritional components and becoming popular as a healthy cereal around the world. It is observed to be a potential source of starch (around 73%) with 18–27% of amylose content [16]. Therefore, high amylose content of tef starch is essential for development of biodegradable edible films [17]. Still, tef-starch films prepared with the blend of camu-camu extract reported by Ju and Song, (2019) and concluded that, tef starch edible film has poor mechanical properties [18]. Similarly, other previous studies also reported that the starch-based edible films has naturally possess poor mechanical properties [19,20]. However, to overcome this problem, appropriate plasticizers and agar should be incorporated in starch-based edible films that can be exhibited ameliorated mechanical properties of films. In order to develop environmentally acceptable packaging materials, agar-agar, a renewable biodegradable polysaccharide, has been used. Agar increases the mechanical and water obstruction qualities of edible films by acting as an excellent cohesive agent in the biopolymer matrix [11,21]. Therefore, to enhance the mechanical attributes of the starch edible films, appropriate agar concentration is very relevant in film forming solution. Furthermore, glycerol can be act as hydrophilic compound and it is helpful in enhancing the film flexibility and mechanical properties of starch edible films [8]. Still now, the tef starch based edible film with better mechanical properties are not reported scientifically.

Hence, the present study was carried out with the objective is to optimization of tef starch, agar and glycerol compositions for edible starch film. In this study also investigated the single and interaction effects of variables on selected mechanical attributes of tef starch based edible films by the help of central composite design of response surface methodology.

2. Materials and methods

2.1. Raw materials

Tef starch was isolated by following the method reported by Bultosa et al. (2002), and used as raw materials for preparation of edible film [22]. The moisture, fat, ash, total protein, amylose and amylopectin content of the isolated tef starch was determined according to the method of AOAC [23]. Food grade glycerol and agar were procured from local laboratory reagent and chemical suppliers.

2.2. Experimental design

Response Surface Methodology was chosen to carry out this experiment by using the Design Expert version 8.0 (Statease, Minneapolis, USA) software. Central Composite Designs (CCD) with 3 variables in 3 levels were used to assess the effect of factors and to optimize the interaction of variables. The independent variables were considered in this study were tef starch, agar and glycerol amounts. Preliminary studies were carried out to determine the maximum and minimum value of factors. Each of the independent variables was coded at three levels between −1 and +1 as listed in Table .1.

2.3. Preparation of edible films

Solution casting method [18] was followed to develop tef starch edible films through varying proportion of tef starch, agar, and glycerol are presented in Table 1. Around 5 ml homologous film forming solution was formulated by dissolving the starch in distilled water. Then the glycerol was added in to the film forming solution and subjected to the heating process on heating plate and gradually

Table 1
Experimental range of independent variables and their levels.

Variable (unit)	Factors X	Levels		
		−1	0	+1
Tef starch (g)	X_1	3	4	5
Agar (g)	X_2	0.3	0.4	0.5
Glycerol (%)	X_3	0.3	0.4	0.5

to 90 °C for 30 min with regular mixing (700 rpm). The film forming solutions was poured in the Petri dish (100 mm diameter and 15 mm deep) carefully and cooled to 25 °C and allowed to at this temperature for 30 min. Further, drying was carried out at 50 °C for 48 h. Finally, the films were cautiously peeled from the dishes and equilibrated at 25 °C at 37% RH for 72 h before determination of properties. The preparation of starch film is illustrated in Fig. 1. All the experiments were triplicated.

2.4. Determination of mechanical properties of prepared films

2.4.1. Tensile and puncture properties

The tensile properties [tensile strength (MPa), elongation at break (%), elastic modulus (MPa), puncture force (N), and puncture deformation (mm)] were studied by using auto tensile tester (XLW (EC), China). Film sample strips with an appropriate size (1.5 × 5 cm) was taken from each prepared tef starch films. Prior to testing, the cross-sectional area of each sample—which is equal to thickness times width—was determined by measuring the thickness of the samples using a micrometre at several capricious positions on the film.

2.5. Characterization of optimized tef starch biodegradable edible film

2.5.1. Scanning electron microscope (SEM)

A scanning electron microscope was used to obtain electron micrographs of the optimized tef starch films (JEOL, Tokyo, Japan) according to the method of Ju and Song [18]. Dried films were positioned on double-sided stick tape put on an aluminum stub, and the film was then covered with gold. An accelerating voltage of 15 kV and magnifications of 1000X and 1500X were used for studying surface of samples.

2.5.2. XRD analysis

The X-ray diffraction spectra of the optimized tef starch based edible film was evaluated using an X-ray diffractometer (XRD-7000, SHIMADZU Corporation, Japan) with previously illustrated method [24]. The necessary parameters such as X-ray, 40 kV, Cu–K radiation, 30 mA, and angle of scanning (2θ) was in the range from 5° to 80° with a scanning speed of 4.0 °C/min. For calculation of the overall area under the curve and the area under each notable peak, the Origin Pro 2018 software program was utilized. The following equation (1) was used to compute the % crystallinity:

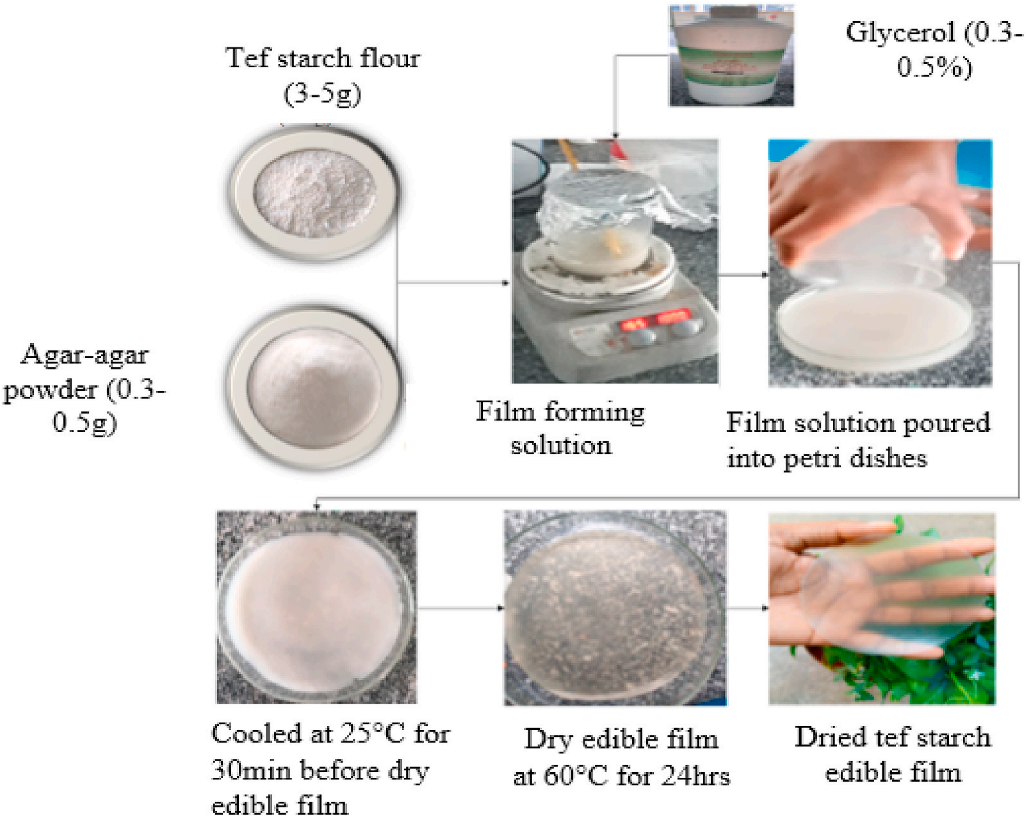

Fig. 1. Graphical description of tef starch-based edible film preparation.

$$\text{Crystallinity } (\%) = \left[\frac{\text{area under peaks}}{\text{Total area}}\right] \tag{1}$$

2.6. Statistical analysis and process optimization

The significant model that explains the link between the independent variables and the response variables was found using the statistical programme Design Expert 8.0 (Stat-ease Inc., Minneapolis, USA). Equation (2) is the result of deriving a quadratic model, which also contains the linear model, from a second-order polynomial model fitted to the CCD.

$$Y_k = \beta_{0+} \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1{}^2 + \beta_{22} X_2{}^2 + \beta_{33} X_3{}^2 + \varepsilon \tag{2}$$

Where Y_k = response variable namely tensile strength (MPa), elongation (%), elastic modulus (MPa), puncture force (N), and puncture deformation (mm); independent coded variables include concentrations of X_1 tef starch (g), X_2 = agar (g), X_3 = glycerol (%).

The test of statistical significance was performed on the total error. Using the analysis of variance (ANOVA) method, the models' suitability was assessed, and their coefficient of determination (R^2) and adjusted R^2 values were calculated. The variables on each response were used to create the F values and R^2 values. By holding two components constant and altering the other two from the fitted polynomial equation, the regression coefficients were utilized to do statistical calculations to create 3D surface plots as a function of the variables on each answer. To comprehend their primary and interacting effects on the dependent variables, these graphs have been created [25]. Finally, the numerical optimization was performed to determine the best composition of the tef starch, agar and glycerol concentrations are required for the edible film with the best mechanical properties.

3. Results and discussions

3.1. Chemical composition of tef starch

The chemical analysis showed that tef starch contained moisture content (8.1 ± 0.06%), total protein content (0.02 ± 0.01%), ash content (0.25 ± 0.01%, dry basis), amylose content (22.2 ± 0.78%, dry basis), and amylopectin content (77.8 ± 0.3%, dry basis), respectively as shown in Table 2.

However, Bultosa, (2008) was also reported that, the composition of the starch influence the quality attributes and functionality of the starch based edible films [26]. Previous study [17] reported similar reports on the composition of the starch from the tef. The amylose amounts in the tef starch influence the film forming attributes and responsible for a film forming capacity [18]. The high amylopectin content of starch significantly affects the mechanical attributes of edible films [27]. Cano et al. [28], reported that, edible films from amylose-rich sources forms crystalline regions during the drying. This higher amylose content gives stiffer, higher resistance to fracture, however, lower stretchable films, with decreased oxygen permeability and higher water binding capacity. However, amylopectin is acts as the plasticizer in the starch based edible films.

The starch-based biodegradable films' hydrophilic nature is its principal drawback. This nature leads to the reduction in film permanency when they exposed to diverse environmental circumstances [29]. Petersson and Stading et al., examined the role of lipids in reducing the hydrophilic nature of starch films; however, as the lipid portion increased in the film forming solution, phase separation was observed and leads to incompatibility of the components [30]. So, lower fat in the tef starch isolated in this study is one of the positive factor for the edible biofilm production. Jiménez et al. (2012) reported the adding the casein (protein) to corn starch decreases the degree of crystallinity in starch films and averts starch recrystallization in the storage [13]. The combination of hydrocolloids and starch modifies the characters of starch films, for instance this can limit the retrogradation [31]. So, lower protein content of the tef starch obtained in present study is good for the starch based edible biofilm preparation. The tef starch considered in this study is best suited for the preparation of starch edible films.

3.2. Mechanical properties of the prepared films

Different Mechanical properties of the starch based edible films are studied and the analysis of variance was presented in Table 3.

3.2.1. Tensile strength

The tensile strength is an imperative character of edible films, it provides an information on the ability of film to endure exterior force and sustain film integrity without breaking. The tensile strength property showed significant ($P < 0.0001$) affect in linear and quadratic terms by the composition of ingredients in the film. The ability of edible films made of starch to tolerate external force and

Table 2
Chemical constitutes of tef starch.

Sample	MC (%)	Fat (%)	TP (%)	Ash (%, dry basis)	Am (%, dry basis)	Ap (%)
Tef starch	8.1 ± 0.06	0.59 ± 0.01	0.02 ± 0.01	0.25 ± 0.01	22.2 ± 0.78	77.8 ± 0.3

MC = Moisture content; TP = Total protein; Am= Amylose; Ap = Amylopectin.
The vales are mean ± Standard deviation of three observations.

Table 3
ANOVA (P-values) for Tensile strength, Elongation at break, and Elastic modulus of tef starch based edible film.

Source	TS	EB	EM	PF	PD
Model	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
A-Tef starch	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B-Agar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
C-Glycerol	<0.0001	0.4314	<0.0001	<0.0001	<0.0001
AB	<0.0001	0.0009	<0.0001	0.6771	<0.0001
AC	<0.0001	0.0010	<0.0001	<0.0001	<0.0001
BC	<0.0001	0.0076	<0.0001	<0.0001	<0.0001
A^2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
B^2	<0.0001	0.8353	<0.0001	<0.0001	<0.0001
C^2	<0.0001	0.0050	0.0002	<0.0001	<0.0001
Lack of fit	0.1526	0.6722	0.0921	0.0942	0.0529

TS = Tensile Strength; EB = Elongation at break; EM = Elastic Modulus; PF= Puncture Force; PD= Puncture Deformation.

preserve their integrity without rupturing depends on their tensile characteristics. This property is very important to determine the suitability of the starch film for packaging applications. The tensile strength of tef starch based biodegradable edible films prepared in this study ranged from 17.97 to 24.25 MPa as shown in Table .4. Fig. 2(a) revealed that, tef starch based edible films with greater concentration of starch and agar demonstrated good tensile strength. The same trends is confirmed in the findings of Wu et al., [32]. The increase in the tensile strength is attributed to the establishment of inter-molecular hydrogen bonds among the agar and tef starch, in addition the compacted structure formations in the biodegradable edible films showed greater tensile strength [33]. According to Tessaro et al., an increased in cassava starch exhibited the higher tensile strength of films [34]. The agar in film forming solution also contributed to the higher tensile strengths. This is attributed to the ability of agar to form more rigid network in the edible films due to the strong interaction of intermolecular hydrogen bonds [35]. Therefore, the current study shown that, amounts of agar and tef starch positively affected the tensile strength of biodegradable edible films.

Fig. 2 (b) exhibited the influence of glycerol and agar on tensile strength of biodegradable edible films. The agar concentration had positive effects on tensile strength of tef starch based biodegradable edible films, but the glycerol was not effective (Fig. 2(c)). As the glycerol concentration was reduced in the mixture, higher tensile strength values were observed [21]. This trend might be attributed to the plasticizers reduced interaction of inter-molecular hydrogen bonding between the starch-agar molecules, therefore, it is reduced the tensile strength of prepared biodegradable edible film in this study. This outcomes is in agreement with the previous findings of Singh et al. (2020) [14], where increasing the amounts of glycerol in film forming solution exhibited the lower tensile strength of edible films. The hydrophilic property of glycerol, which can hold more water molecules and has a stronger plasticizing impact, is the factor for its detrimental effects on tensile strength [36]. The tensile strength properties of the of tef starch edible film prepared in this study are comparable with those of the other starch films, such as pearl millet starch edible films [14], agar based edible films [37].

3.2.2. Elongation at break

The elongation at break property showed significantly ($P < 0.0001$) affected in linear and quadratic terms by the composition of tef starch, agar, and glycerol in the film. The elongation at break shows how long a film can be extended from the beginning to the break.

Table 4
The mechanical properties of the edible film as the composition at various starch, Agar and Glycerol concentrations.

Run	Tef starch (g)	Agar (g)	Glycerol (%)	TS (MPa)	EB (%)	EM (MPa)	PF (N)	PD (mm)
1	4	0.4	0.4	24.01	1.58	84.90	7.26	14.23
2	3	0.3	0.5	19.31	1.81	18.98	2.55	14.95
3	5	0.3	0.3	20.20	1.60	83.79	12.65	10.95
4	5	0.4	0.4	23.4	1.4	75.66	11.68	12.02
5	4	0.3	0.4	21.98	1.90	75.56	6.99	14.46
6	4	0.5	0.4	24.01	1.37	107.54	11.06	12.76
7	4	0.4	0.4	24.01	1.63	84.84	7.41	14.24
8	5	0.5	0.5	20.16	1.40	77.06	14.02	13.2
9	4	0.4	0.4	24.01	1.66	84.90	7.26	14.19
10	4	0.4	0.5	21.89	1.72	77.98	4.46	15.8
11	3	0.3	0.3	17.97	2.03	17.58	10.32	14.05
12	5	0.5	0.3	24.25	1.21	108.6	15.02	9.59
13	3	0.5	0.5	19.52	1.35	61.02	8.20	13.02
14	3	0.5	0.3	20.4	1.41	81.00	12.38	11.27
15	4	0.4	0.4	24.01	1.65	83.75	7.25	14.2
16	5	0.3	0.5	18.82	1.60	74.46	8.56	13.95
17	3	0.4	0.4	21.88	1.56	33.96	7.4	13.4
18	4	0.4	0.4	23.89	1.59	84.97	7.33	14.22
19	4	0.4	0.3	23.12	1.72	94.98	8.9	13.52
20	4	0.4	0.4	24.01	1.69	84.97	7.26	14.24

Where, TS = Tensile strength; EB = Elastic modulus; EM = Elongation at break; PF= Puncture force; PD = Puncture deformation.

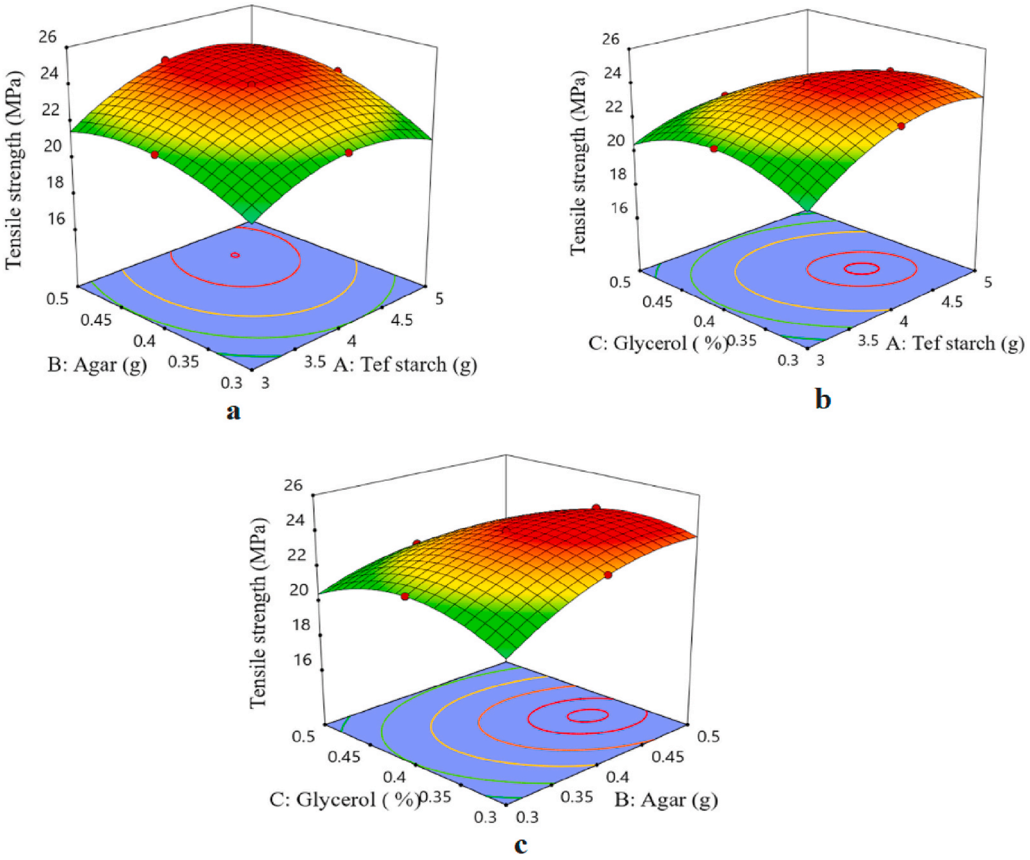

Fig. 2. Three-dimensional surface plot revealing the effects of tef starch (g), agar (g), and glycerol (%) on Tensile Strength (Mpa) of prepared edible film. Where; a). Effect of Agar (g) and Tef starch (g) levels on the Tensile Strength(Mpa) of prepared edible film; b). Effect of Glycerol (%) and Tef starch (g) levels on the tensile strength (Mpa) of prepared edible film; c). Effect of the Glycerol (%) and Agar (g) on Tensile Strength (Mpa) of prepared edible film.

This factor represents the flexibility and stretchability of the films. The flexibility of the biodegradable packaging films determines intended application and succeeding transportation, handling and storage of packed foods. The elongation at break of tef starch based edible film ranged from 1.21 to 2.03% as shown in Table 4. Fig. 3(a) showed the significant effects of agar and tef starch concentration on the elongation at break value of the prepared films. This study showed that, as the tef starch ratio increased the elongation at break of biodegradable edible films were decreased. In contrast, as agar ratio increased the intermolecular hydrogen bonds among polysaccharide chains are reduced and increase their flexibility, then increased the elongation [33]. In general elongation at break reduced as the agar portion increased [38]. This trend is similar with the previous findings of potato starch films reported by Wu et al., [32]. They reported that, the proportions of agar in the film forming solution increased the elongation at break decreased gradually, while the tensile strength was increased. This reduction in the elongation at break is attributed to the three-dimensional linkages and development of intermolecular hydrogen bonds among agar and starch restrained the mobility of polysaccharide chains [33].

Fig. 3(b) revealed the effects of agar and glycerol concentrations on elongation at break of tef based biodegradable edible films. In this study identified that, as the glycerol concentration raised in the film forming solution the increase in elongation at break observed, while decreased the tensile strengnth of the prepaed edibe film (Fig. 3(c)). The results of present study are confirmed with the previous findings [32], where the concentaration of glycerol raised the elongation at break of potato starch film. This increase in the elongarion at break of the edible film is attributed to the hydrophilic nature of starch–agar polymer chains. The intensity of the intermolecular forces that improve mobility between molecular chains and increase elongation are weaker when glycerol levels are increased [39]. Similerly, Banna et al. [40], reported in increase of elongation at break of sugar palm starch edible film when plasticizer amounts increased. This trend might be observed due to the decrease in plasticizer leads to the inteaction of intermolucelar hydrogen bonding between polymer molucules.

3.2.3. Elastic modulus

An elastic material's elastic modulus, which can be used for correct characterization and development, assesses the toughness of the material. The elastic modulus of the prepared edible biofilm showed significant ($P < 0.0001$) affect in linear and quadratic terms by the composition of tef starch, agar, and glycerol. The elastic modulus of tef starch based edible film ranged from 17.58 to 108.69 MPa

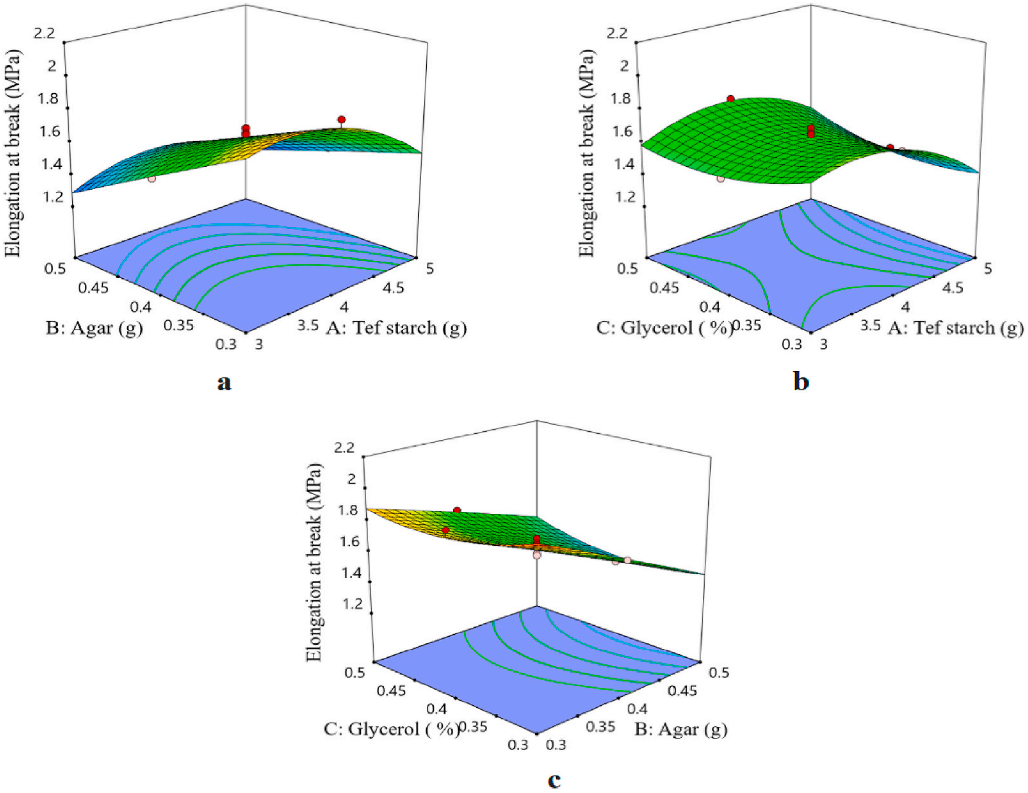

Fig. 3. Three-dimensional surface plots revealing the effects of Tef starch (g), Agar (g), and Glycerol (%) on Elongation at break(Mpa). Where; a). Effect of Agar (g) and Tef starch (g) levels on the Elongation at Break (Mpa) of prepared edible film; b). Effect of Glycerol (%) and Tef starch (g) levels on the Elongation at Break(Mpa) of prepared edible film; c). Effect of the Glycerol (%) and Agar (g) on Elongation at Break (Mpa)of prepared edible film.

(Table 4). Fig. 4(a) showed the effects of agar and glycerol on the elastic modulus of biodegradable edible films. Addition of agar in edible films had positive effects on elastic modulus. The present study showed that, the higher values of elastic modulus was examined for biodegradable edible films with lower glycerol content. This lower elastic modulus may be due to the intermolecular interactions between polysaccharide chains in dried films [33]. The same trends of the present study results also reported by the Atef et al. [41], on agar-based nanocomposite film and Araujo-farro et al. [42], on quinoa based starch edible films.

Glycerol highly determine the elastic modulus of starch film, with a dismissive effect on response. From the study it is observed that, the lower elastic modulus values of biodegradable edible films with higher glycerol content. This could be due to the plasticizing effects of glycerol, which alter the structure of the starch network, weaken the matrix, and reduce the attractive interactions between the polymer chains [43]. Prakash Maran et al. [33] showed that the elastic modulus of edible films (maize starch) reduced with increasing the glycerol content, this was attributed to the formation of discontinuities in the polymer matrix in the dried film. The decrease in the elastic modulus of agar-based nanocomposite film with increasing concentration of the nanocrystalline cellulose was reported by the Atef et al. [41] while without nanocrystalline cellulose elastic modulus increased. The increase in elastic modulus of tef starch based biodegradable edible films with increased agar and tef starch contents showed in Fig. 4(b). This trend might be due to the formation of strong interaction among the intermolecular forces between agar-starch molecules (Fig. 4(c)). Similar elastic modulus values of the starch edible films also reported by the Luchese et al., [34]. As upsurge in the starch encouraged in development of the resistance properties at break and film stiffness.

3.2.4. Puncture force

The greatest force necessary to cut through a substance is called the puncture force. The puncture force property showed significantly ($P < 0.0001$) affected in linear and quadratic terms by the composition of tef starch, agar, and glycerol in the film. The puncture force of tef starch edible film varied from 2.55 N to 15.02 N as shown in Table 4. Fig. 5(a) showed the influence of tef starch and agar on the puncture force of edible films. Puncture force of tef starch edible film is increased with raising the concentration of tef starch and agar. A similar fashion was given by Prakash Maran et al. [33], for maize starch based edible films, when raise in maize concentration, puncture force also reported higher. Prakash Marana et al. [33], also showed that, water vaporizes during the film-forming solution's drying phase and allowing the formation of starch network. At this juncture, closer starch chains caused by higher starch concentrations may make a matrix with a higher starch content per unit area easier to form. This higher concentrations of the starch per unit

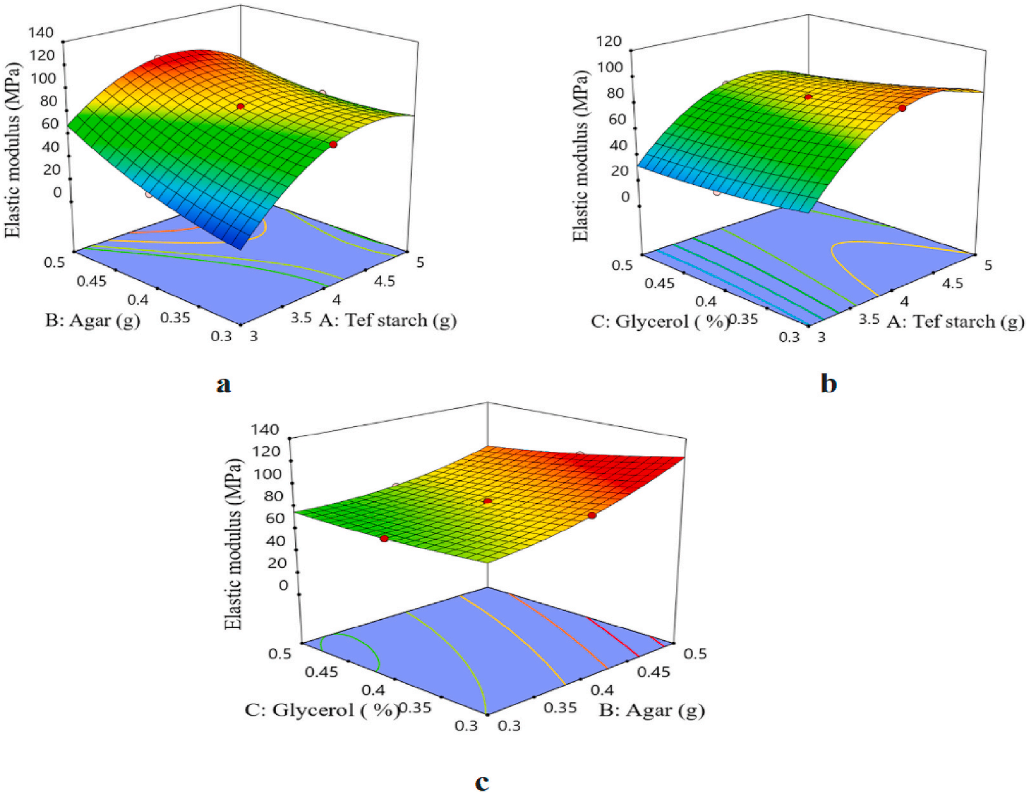

Fig. 4. Three-dimensional surface plots revealing the effects of tef starch (g), agar (g), and glycerol (%) on Elastic modulus (Mpa) of prepared edible film. Where; a). Effect of Agar (g) and Tef starch (g) levels on the Elastic modulus (Mpa) of prepared edible film; b). Effect of Glycerol (%) and Tef starch (g) levels on the Elastic modulus (Mpa) of prepared edible film; c). Effect of the Glycerol (%) and Agar (g) on Elastic modulus (Mpa) of prepared edible film.

area increases the force required to puncture.

Direct interactions and the closeness of starch chains were reduced when a plasticizer was added to the film-forming solution. Thus, while under stress, starch chain oscillations were expedited and the puncture force was reduced. The three-dimensional network structure made by the tangling of agar strands expanded together with the increase in agar concentration [33].

Fig. 5 (b & c) showed the effects of glycerol, agar and tef starch on the puncture force of films. The increasing of glycerol concentration was negatively affected the puncture force of tef starch edible films. This study cleared that addition of higher glycerol concentration in tef starch edible films decreased the puncture force. Saberi et al. [44], showed that, the puncture force values of tef starch edible film were decreased with increasing the glycerol concentration. The effect of glycerol is well-known in starch based edible biofilms. When glycerol is introduced into the starch network, intermolecular interactions are reduced and polymer chain mobility is increased [45]. Gontard et al. [46], also reported linear reduction of the puncture force in edible films from 1.9 to 0.3 N as the glycerol concentration increased in film forming solution.

3.2.5. *Puncture deformation*

The puncture deformation is the important parameter to apprehended the mechanical resistibility of film. The puncture deformation property showed significant ($P < 0.0001$) affect in linear and quadratic terms by the ratios of tef starch, agar, and glycerol in the film. Puncture deformation of prepared tef starch based edible film ranged from 9.59 to 14.95 mm as shown in Table 4. The effects of tef starch, agar and glycerol on the puncture deformation was showed in Fig. 5 (a, b and c). From this study it is observed that, puncture deformation raised linearly as the glycerol concentration increased and reduced in tef starch and agar amounts as shown in Fig. 6 (b & c). Fig. 6(c) showed that, puncture deformation of tef starch edible film decreased when the agar and tef starch concentration increased. Similarly, glycerol concentration was also positively affected the puncture deformation. This trend is observed similar with the previous findings [33].

Due to the formation of hydrogen bonds between the hydroxyl groups of starch and glycerol, the glycerol molecules reduce the tight binding between the starch macromolecules. This causes the starch films' elasticity to rise. Furthermore, raising in the starch amounts in film forming solution also enlarged the puncture force. According to Prakash Maran et al. [33], reported the tapioca starch films puncture deformation elevated due to the enhanced suppleness of polymers in the occurrence of glycerol. Sobral et al. [45], also reported that, puncture deformation of gelatin film upsurges by raising in plasticizer amounts. This trend may be attributed to the raise

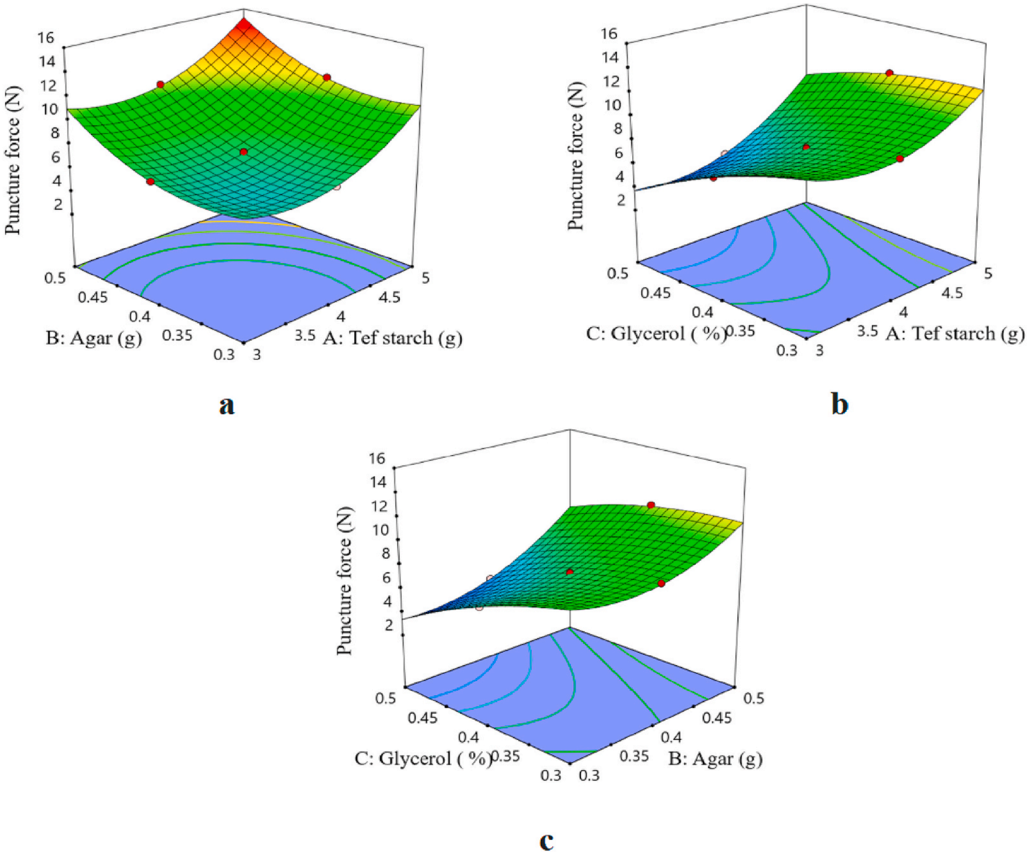

Fig. 5. Three-dimensional surface plots revealing the effects of tef starch (g), agar (g), and glycerol (%) on Puncture Force (N) of prepared edible film. Where; a). Effect of Agar (g) and Tef starch (g) levels on the Puncture Force (N) of prepared edible film; b). Effect of Glycerol (%) and Tef starch (g) levels on the Puncture Force (N) of prepared edible film; c). Effect of the Glycerol (%) and Agar (g) on Puncture Force (N) of prepared edible film.

in flexibility of macromolecules and this also leads to the less dense matrix films [33]. The decrease in the puncture force and the increase of puncture deformation were accredited to the amalgamation of plasticizers, and water molecules absorbed by the samples. This is a very general spectacle of edible films reported in different scientific studies [33,45,46].

3.3. Model for prediction of tef starch edible film properties

The present experiments were carried out according to response surface methodology in central composite design and total number of experiments were 20. The levels of factors, tef starch (X_1), agar (X_2), and glycerol (X_3) and the effects of interaction on tensile strength, elongation at break, elastic modulus, puncture force, and puncture deformation were determined by response surface methodology in central composite design. The data obtain was fitted to the several models (linear, interactive, quadratic and cubic) to attain regression model. Two different tests namely the sequential model sum of squares, and model summary statistics were carried out in this study to decide the adequacy of models. The quadratic model possessed the greatest "R^2" value for various responses and also generated the regression equations shown in Table 5.

The fit summary findings showed that the quadratic model was the most intriguing of all the models of comparison. In ANOVA, a quadratic regression model showed F-value of 84.98, $P < 0.0001$ for Tensile strength, F-value of 0.7363, $P < 0.0001$ for elongation at break, F-value of 11597.43, and $P < 0.0001$ for elastic modulus, F-value of 186.12, $P < 0.0001$ for puncture force, F-value of 41.60, $P < 0.0001$ for puncture deformation as shown in Table 5.

3.4. Optimization and validation of the process parameters

In the present study, obtained second order polynomial models were conceded for each response parameters and also find the optimum conditions. The desirability function method was employed for optimization of the various responses. The optimization target was to minimize solubility, and elongation at break while maximizing tensile strength and elastic modulus. The numerical optimization provided that, 5g of tef starch, 0.4 g of agar and glycerol concentration of 0.30% as the optimum components. The starch based edible biofilm prepared with optimum compositions will provide (system generated) the film with 23.18 MPa of tensile strength,

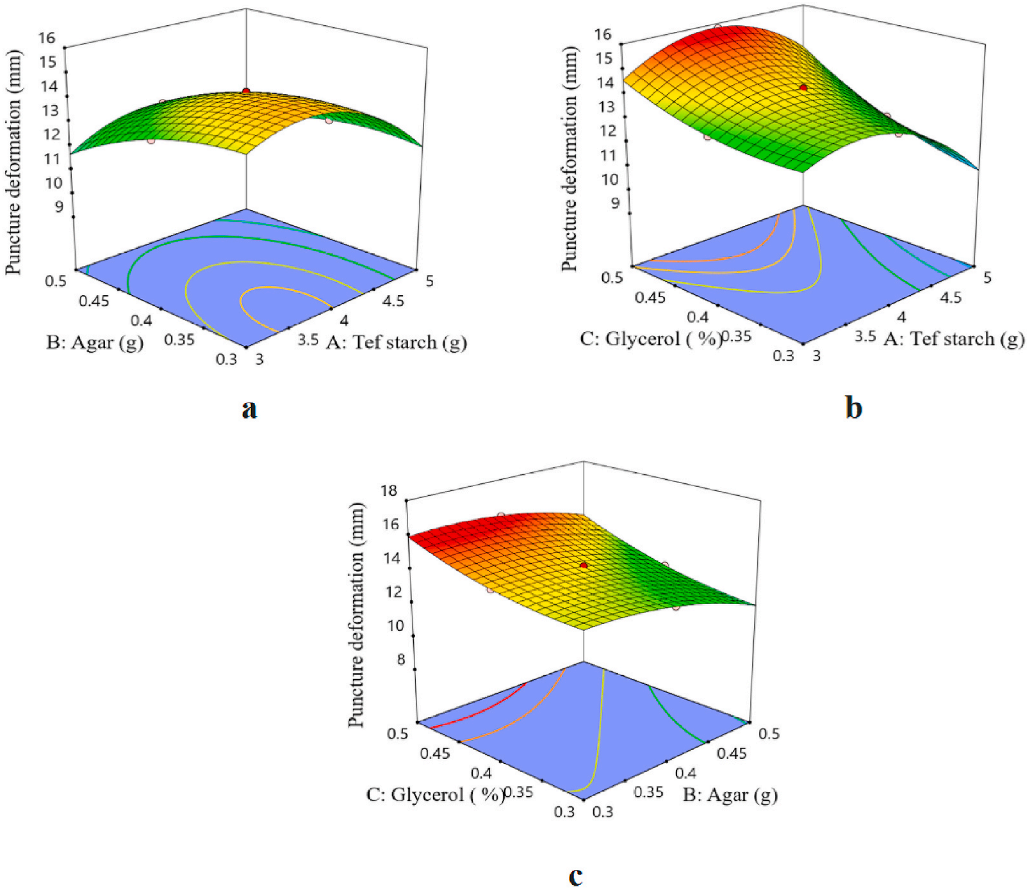

Fig. 6. Three-dimensional surface plots revealing the effects of tef starch (g), agar (g), and glycerol (%) on Puncture Deformation (mm) of prepared edible film. Where; a). Effect of Agar (g) and Tef starch (g) levels on the Puncture Deformation (mm)of prepared edible film; b). Effect of Glycerol (%) and Tef starch (g) levels on the Puncture Deformation (mm) of prepared edible film Puncture Deformation (mm); c). Effect of the Glycerol (%) and Agar (g) on Puncture Deformation (mm)of prepared edible film.

Table 5
The regression equation and R^2 values of tensile strength, elongation at break, elastic modulus, puncture force, and puncture deformation.

Property	Regression Equation	R^2 Value
TS	$+ 24.00 + 0.7752X_1 + 1.01X_2 - 0.6229X_3 + 0.3442X_1X_2 - 0.7408X_1X_3 - 0.6150X_2X_3 - 1.38X_1{}^2 - 1.02X_2{}^2 - 1.51X_3{}^2$	0.9995
EB	$+ 1.64 - 0.0964X_1 - 0.2189X_2 - 0.0098X_3 + 0.0620X_1X_2 + 0.0610X_1X_3 + 0.0445X_2X_3 - 0.1614X_1{}^2 - 0.0049X_2{}^2 + 0.0816X_3{}^2$	0.9810
EM	$84.45 + 20.71X_1 + 16.49X_2 - 7.65X_3 - 9.75X_1X_2 - 2.80X_1X_3 - 5.46X_2X_3 - 29.21X_1{}^2 + 7.53X_2{}^2 + 2.46X_3{}^2$	0.9995
PF	$+ 7.33 + 2.11X_1 + 1.96X_2 - 2.15X_3 + 0.0149X_1X_2 + 0.8564X_1X_3 + 0.8354X_2X_3 + 2.17X_1{}^2 + 1.66X_2{}^2 - 0.6854X_3{}^2$	0.9995
PD	$+ 14.21 - 0.6980X_1 - 0.8519X_2 + 1.15X_3 + 0.3250X_1X_2 + 0.4950X_1X_3 + 0.1825X_2X_3 - 1.48X_1{}^2 - 0.5799X_2{}^2 + 0.4701X_3{}^2$	0.9997

Where, X1 = Tef starch; X2 = Agar; X3 = Glycerol; TS = Tensile strength; EB = Elastic modulus; EM = Elongation at break; PF= Puncture force; PD = Puncture deformation.

1.42% of elongation at break, 88.67 MPa of elastic modulus, 10.65 N of puncture force, and 12.65 mm of puncture deformation.

This optimal condition was used to validated experimentally in order to confirm the adequacy of a model. The optimized value from the developed empirical model equations were used to carried out triplicate experiments and to compare the experimental outcomes with the predicted values. The teff based biofilm from the optimized blending of ingredients showed (measured) 23.18 MPa for tensile strength, 1.42% elongation at break, 88.76 MPa elastic modulus, 10.60 N puncture force, 12.62 mm puncture deformation. The mechanical characteristics that were discovered to be in conformity with the projected values made it abundantly evident that the constructed quadratic models were suitable. It should be emphasized that these optimal values are valid within the required range of process parameters given the results of the confirmation studies, which demonstrate the applicability of the generated quadratic models.

3.5. Characterization of optimized tef starch edible film

3.5.1. Morphological properties

The scanning electron microscope images of the optimized tef starch biodegradable film is showed in Fig. 7. The tef starch edible film had some pores on the surface, aggregation of starch, and agar granules are also observed on the surface of edible film. The optimized edible film had rough, coarser, and irregular surface. Similar trend is observed with previous findings by Madera-santana et al. [38],. Ju and Song [18], showed that, scanning electron microscope of tef starch edible film has smooth and homogenous surface, while in the present study the scanning electron microscope of tef starch has not uniform smooth surface due to increasing the concentration of agar in film forming solution. Comparable finding is observed with earlier findings of Wu et al. [32], the raise in agar had showed substantial influence on the microstructure of potato starch film.

Strong molecular interactions between tef starch, agar, and glycerol and the development of a continuous phase of polymeric matrix are responsible for these microscopic structural characteristics. Its good mechanical qualities, notably tensile strength, may be due to the compacted structure of composite film with better structural solidity than stated film made of pearl millet starch [47].

3.5.2. XRD analysis of the optimized tef starch based edible film

A study of X-ray diffractograms was done to assess the amorphous-crystalline structure, which is categorized by strong peaks related with crystalline diffraction and an amorphous zone [48]. The edible biofilm from optimized formula showed semi-crystalline in structure. The XRD spectra of optimized tef starch based edible film shown in Fig. 8. The XRD of tef starch revealed the three main diffraction peaks at $2\,\theta = 17.14^\circ$, 20°, and 22.04°. This result is disclosed that the incorporation of agar enhanced the crystallinity of optimized films and interaction among agar and tef starch matrix [37]. Similar XRD result was reported by Ibrahim et al. [49], on corn starch-based films as affected by different concentrations of the plasticizers. Wu et al. [32], also showed that, incopropration of agar improved the microstructure of potato starch edible films. Tef starch edible film was showed strong peak intensity (22.04°) due to the development of tough intereaction of hydrogen bonding among agar and stach molecules. The peaks at 22.04° became broader reveals the effective compatability between agar and starch [49]. Starch film from cassava starch based film reported higher peak intensity owing to the formation of intermolecular hydrogen bond than the reported study [50].

On the other hand, optimized tef starch edible films with plasticizer (glycerol) content revealed lower peak intensities or crystallinity which indicates a superior amorphous zone and minor peak intensity. Similar result was also described by Edhirej et al. [50], for cassava starch edible films by plasticizier. García et al. [48], also showed that the maize starch films with glycerol provides higher peak intensities or crystallinity and lower amorphous region. However, the presence of plasticizier (glycerol) in starch film formulation was not significantly affect the XRD pattern of optimized film. This result is close agreement with the previous findings of starch based films [48]. Relative crystallinity of optimized tef starch based edible film was 34.18%. The concentration of plasticizier significantly affected the peak intensity of optimized tef starch film. Corn starch film had higher degree of crystallinity when increasing concentration of plasticizier [49].

4. Conclusion

Tef starch edible films were homogenous, flexible, transparent, and biodegradable eco-friendlily packaging materials. The result exhibited that, the plasticizer (glycerol) was the most relevant parameters influencing the mechanical properties of the tef starch edible films. On the other hand, agar incorporation in edible films was enhanced the mechanical properties of tef starch based edible films due to the formation of strong interaction of inter-molecular hydrogen bonding between starch-agar molecules. The prepared film showed the tensile strength of 17.97–24.25 MPa, elongation break of 1.21–2.03%, elastic modulus of 17.58–108.69 MPa, puncture force of 2.55–15.02 N, puncture formation of 9.59–14.95 mm. The statistical analysis revealed a high coefficient of determination value (R^2) for tensile strength (0.9995), for elongation at break (0.9810), for elastic modulus (0.9995), for puncture force (0.9995), and for puncture deformation (0.9997). Therefore, it is confirming the fit of the second order polynomial regression model with actual data. The numerical optimization provided that, 5g of tef starch, 0.4 g of agar and glycerol concentration of 0.30% provides starch based edible biofilm with 23.18 MPa of tensile strength, 1.42% of elongation at break, 88.67 MPa of elastic modulus, 10.65 N of puncture force, and 12.65 mm of puncture deformation. The optimized tef starch edible film exhibited lower elongation at break, and puncture deformation but higher tensile strength, elastic modulus and puncture deformation. The increase in agar had significant effects on the microstructure of tef starch edible film. The XRD results concluded that the presence of agar in starch film forming solution was improved the crystallinity of film. Finally, from this study can conclude that composite biodegradable edible film from tef starch with agar exhibited good mechanical properties. So, tef starch based edible films prepared in this study can be used in food industry for food packaging or coating applications.

Author contribution statement

Kenenisa Dekeba Tafa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Neela Satheesh: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Worku Abera: Performed the experiments; Contributed reagents, materials, analysis tools or data.

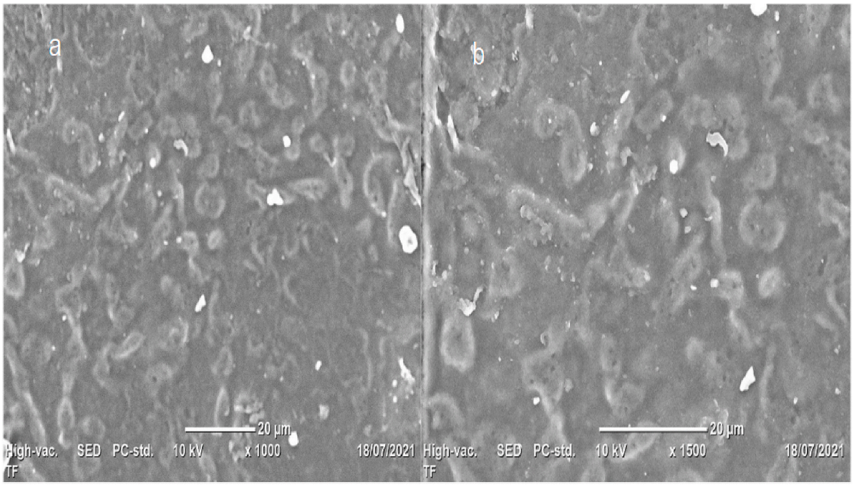

Fig. 7. Scanning electron micrograph surface of optimized tef starch based edible film. Where a. Magnification at 1000×, b. Magnification at 1500X.

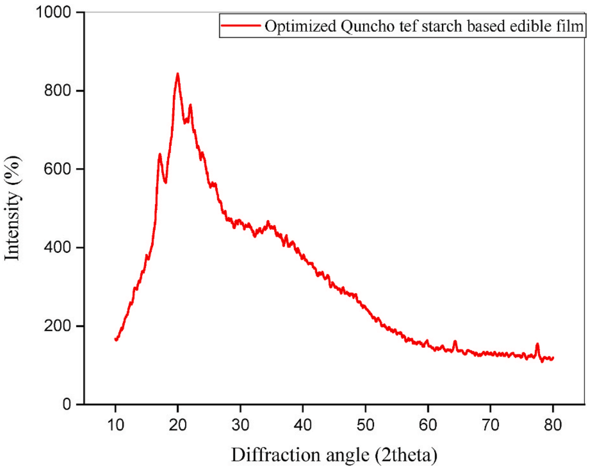

Fig. 8. XRD curves of optimized tef starch edible film.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no competing interests.

References

[1] Plastic Europe, Plastics – the Facts 2020, an Analysis of European Plastics Production, Demand and Waste Data, 2020.
[2] J. Holica, Starch fi lms as an environmentally friendly packaging material : printing performance, J. Clean. Prod. 276 (2020), https://doi.org/10.1016/j.jclepro.2020.124265.
[3] P. Paj, I. Przetaczek-ro, Development and physicochemical , thermal and mechanical properties of edible fi lms based on pumpkin , lentil and quinoa starches, Int. J. Biol. Macromol. 138 (2019) 441–449, https://doi.org/10.1016/j.ijbiomac.2019.07.074.

[4] F. Matta, S. Maria, T. Caon, J. Ignacio, L. Helena, I. Mei, Postharvest Biology and Technology Edible fi lms and coatings based on starch/gelatin : film properties and effect of coatings on quality of refrigerated Red Crimson grapes, Postharvest Biol. Technol. 109 (2015) 57–64, https://doi.org/10.1016/j.postharvbio.2015.05.015.

[5] B. Iamareerat, M. Singh, M. Bilal, Reinforced cassava starch based edible film incorporated with essential oil and sodium bentonite nanoclay as food packaging material, J. Food Sci. Technol. (2018), https://doi.org/10.1007/s13197-018-3100-7.

[6] S. Clotildes, S. Rodrigues, A. Suime, L. Hecker, D. Carvalho, T. Soares, R. Barbosa, Morphological , structural , thermal properties of a native starch obtained from babassu mesocarp for food packaging application, J. Mater. Res. Technol. 9 (2020) 15670–15678, https://doi.org/10.1016/j.jmrt.2020.11.030.

[7] Y.Y. Tye, C.K. Saurabh, L.C. Peng, Biodegradable polymer films from seaweed polysaccharides : a review on cellulose as a reinforcement material, Express Polym. Lett. 11 (4) (2017) 244–265, https://doi.org/10.3144/expresspolymlett.2017.26.

[8] A.M. Youssef, S.M. El-sayed, Bionanocomposites materials for food packaging applications : concepts and future outlook, Carbohydr. Polym. 193 (2018) 19–27, https://doi.org/10.1016/j.carbpol.2018.03.088.

[9] K. Chi, H. Wang, J.M. Catchmark, Sustainable starch-based barrier coatings for packaging applications, Food Hydrocolloids 103 (2020), 105696, https://doi.org/10.1016/j.foodhyd.2020.105696.

[10] S. Haider, T.M. Ali, Hasnain, M. Ashaikh, Bid, Physical, thermal, mechanical and barrier properties of pearl millet starch films as affected by levels of acetylation and hydroxypropylation, Int. J. Biol. Macromol. 11 (2018) 135, https://doi.org/10.1016/j.ijbiomac.2018.11.135.

[11] D. Šuput, V.L. Ć, A.J. Ć, L.L. Ć, L. Pezo, The effect of sorbitol content on the characteristics of starch based edible films, J. Process. Energy Agric. 17 (2013) 106–109.

[12] V. Prakash Maran, K. Sivakumar, S.K. Thirugnanasambandham, Modeling and analysis of film composition on mechanical properties of maize starch based edible films, Int. J. Biol. Macromol. 62 (2013) 565–573, https://doi.org/10.1016/j.ijbiomac.2013.09.027.

[13] A. Jimenez, M.J. Fabra, P. Talens, A. Chiralt, Edible and biodegradable starch films: a review, Food Bioprocess Technol. 5 (2012) 2058–2076, https://doi.org/10.1007/s11947-012-0835-4.

[14] K. Singh, L. Sharma, M. Kaur, R. Kaur, Physical , structural and thermal properties of composite edible fi lms prepared from pearl millet starch and carrageenan gum : process optimization using response surface methodology, Int. J. Biol. Macromol. 143 (2020) 704–713, https://doi.org/10.1016/j.ijbiomac.2019.09.111.

[15] Y. Zhang, Comparison of physicochemical and mechanical properties of edible fi lms made from navy bean and corn starches, J. Sci. Food Agric. (2020), https://doi.org/10.1002/jsfa.10772.

[16] H. Shumoy, K. Raes, Tef : the rising ancient cereal : what do we know about its nutritional and health benefits, Plant Foods Hum. Nutr. (2017), https://doi.org/10.1007/s11130-017-0641-2.

[17] G. Chinnasamy, K. Dekeba, V. Prabhu, Physicochemical properties of tef starch : morphological , thermal , thermogravimetric , and pasting properties, Int. J. Food Prop. 25 (2022) 1668–1682, https://doi.org/10.1080/10942912.2022.2098973.

[18] A. Ju, K. Bin Song, Development of teff starch films containing camu-camu (*Myrciaria dubia* Mc . Vaugh) extract as an antioxidant packaging material, Ind. Crop. Prod. 141 (2019), 111737, https://doi.org/10.1016/j.indcrop.2019.111737.

[19] A. Farahnaky, B. Saberi, M. Majzoobi, Effect of Glycerol on Physical and Mechanical Properties of Wheat Starch Edible Films, vol. 44, 2013, pp. 176–186, https://doi.org/10.1111/jtxs.12007.

[20] R. Sothornvit, M. Krochta, Plasticizers in edible films and coatings, Innov. Food Packaging 1–12 (2005).

[21] R. Arham, M.T. Mulyati, M. Metusalach, S. Salengke, Physical and mechanical properties of agar based edible film with glycerol plasticizer, Int. Food Res. J. 23 (2016) 1669–1675.

[22] Bultosa, J.R.N. Taylor, Z. Trotter, Physico-chemical characterization of grain tef [*Eragrostis tef* (zucc.) trotter], Starch 54 (2002) 461–468.

[23] AOAC, Association of official analytical chemists, in: seventeenth ed.17Official Methods of Analysis of AOAC Internacional, Gaithersburg, 2007, pp. 2–3.

[24] H. Huang, Q. Jiang, Y. Chen, X. Li, X. Mao, X. Chen, L. Huang, W. Gao, Preparation, physico-chemical characterization and biological activities of two modified starches from yam (Dioscorea Opposita Thunb.), Food Hydrocolloids 55 (2016) 244–253, https://doi.org/10.1016/j.foodhyd.2015.11.016.

[25] T.P. Singh, M.K. Chatli, J. Sahoo, Development of chitosan based edible films : process optimization using response surface methodology, J. Food Sci. Technol. (2014), https://doi.org/10.1007/s13197-014-1318-6.

[26] G. Bultosa, B.R. Hamaker, J.N. BeMiller, An SEC− MALLS study of molecular features of water-soluble amylopectin and amylose of tef [Eragrostis tef (Zucc.) Trotter] starches, Starch Staerke 60 (1) (2008) 8–22.

[27] E. Šárka, V. Dvoracek, Waxy starch as a perspective raw material (a review), Food Hydrocolloids 69 (2017) 402–409, https://doi.org/10.1016/j.foodhyd.2017.03.001.

[28] A. Cano, A. Jimenez, M. Chafer, C. Gonzalez, A. Chiralt, Effect of amylose:amylopectin ratio and rice bran addition on starch films properties, Carbohydr. Polym. 111 (2014) 543–555, https://doi.org/10.1016/j.carbpol.2014.04.075.

[29] G.K. Moates, P.M. Forssell, S.H.D. Hulleman, R. Parker, Ageing of rubbery thermoplastic barley and oat starches, Carbohydr. Polym. 39 (1999) 43–51.

[30] M. Petersson, M. Stading, Water vapour permeability and mechanical properties of mixed starch-monoglyceride films and effect of film forming conditions, Food Hydrocolloids 19 (2005) 123–132, https://doi.org/10.1016/j.foodhyd.2004.04.021.

[31] M. Yoshimura, T. Takaya, K. Nishinari, Effects of xyloglucan on the gelatinization and retrogradation of corn starch as studied by rheology and differential scanning calorimetry, Food Hydrocolloids 13 (1999) 101–111, https://doi.org/10.1016/S0268-005X(98)00075-7.

[32] Y. Wu, F. Geng, P.R. Chang, J. Yu, X. Ma, Effect of agar on the microstructure and performance of potato starch film, Carbohydr. Polym. 76 (2009) 299–304, https://doi.org/10.1016/j.carbpol.2008.10.031.

[33] V. Prakash Marana, R. Sivakumara, V.P.I. Sridharb, Development of model for mechanical properties of tapioca starch based edible films, Ind. Crop. Prod. 42 (2013) 159–168, https://doi.org/10.1016/j.indcrop.2012.05.011.

[34] C.L. Luchese, J.C. Spada, I.C. Tessaro, Starch content affects physicochemical properties of corn and cassava starch-based films, Ind. Crop. Prod. 109 (2017) 619–626.

[35] P. Wongphan, N. Harnkarnsujarit, Characterization of starch , agar and maltodextrin blends for controlled dissolution of edible fi lms, Int. J. Biol. Macromol. 156 (2020) 80–93, https://doi.org/10.1016/j.ijbiomac.2020.04.056.

[36] R. Thakur, B. Saberi, P. Pristijono, C.E. Stathopoulos, J.B. Golding, C.J. Scarlett, M. Bowyer, Q.V. Vuong, Use of response surface methodology (RSM) to optimize pea starch – chitosan novel edible film formulation, J. Food Sci. Technol. (2017), https://doi.org/10.1007/s13197-017-2664-y.

[37] X. Wang, C. Guo, W. Hao, N. Ullah, L. Chen, Z. Li, X. Feng, Development and characterization of agar-based edible fi lms reinforced with nano-bacterial cellulose, Int. J. Biol. Macromol. 118 (2018) 722–730, https://doi.org/10.1016/j.ijbiomac.2018.06.089.

[38] T.J. Madera-santana, Y. Freile pelegrin, J.A. Azamar-barrios, Physicochemical and morphological properties of plasticized poly(vinyl alcohol)–agar biodegradable films, Int. J. Biol. Macromol. 69 (2014) 176–184, https://doi.org/10.1016/j.ijbiomac.2014.05.044.

[39] Sari Katili, S.I. Bayu Tri Harsunu, The effect of concentration of glycerol plasticizer and chitosan composition in solution on physical properties of edible film of chitosan, J. Technol. 6 (2013) 29–38.

[40] H. Banna, M. Zukaul, A. Bin, H. Susan, A. Bin Imran, E ff ects of plasticizers and clays on the physical, chemical, mechanical, thermal, and morphological properties of potato starch-based nanocomposite films, ACS Omega (2020) 17543–17552, https://doi.org/10.1021/acsomega.0c02012.

[41] M. Atef, M. Rezaei, R. Behrooz, Preparation and characterization agar-based nanocomposite film reinforced by nanocrystalline cellulose, Int. J. Biol. Macromol. 70 (2014) 537–544, https://doi.org/10.1016/j.ijbiomac.2014.07.013.

[42] P.C. Araujo-farro, G. Podadera, P.J.A. Sobral, F.C. Menegalli, Development of films based on quinoa (Chenopodium quinoa , Willdenow) starch, Carbohydr. Polym. 81 (2010) 839–848, https://doi.org/10.1016/j.carbpol.2010.03.051.

[43] M. Augusto, R. Sanches, C. Camelo-silva, L. Tussolini, M. Tussolini, R. Carlos, P. Becker, Development , characterization and optimization of biopolymers films based on starch and flour from jabuticaba (Myrciaria cauliflora) peel, Food Chem. 343 (2021), 128430, https://doi.org/10.1016/j.foodchem.2020.128430.

[44] B. Saberi, Q.V. Vuong, S. Chockchaisawasdee, J.B. Golding, C.J. Scarlett, C.E. Stathopoulos, Mechanical and physical properties of pea starch edible films in the presence of glycerol, J. Food Process. Preserv. 40 (2016) 1339–1351, https://doi.org/10.1111/jfpp.12719.

[45] P.J.A. Sobral, F.C. Menegalli, M.D. Hubinger, M.A. Roques, Mechanical , water vapor barrier and thermal properties of gelatin based edible ® lms, Food Hydrocolloids 15 (2001) 423–432.

[46] N. Gontard, S. Guilbert, J. Cuq, Edible wheat gluten films : influence of the main process variables on film properties using response surface methodology, J. Food Sci. 57 (1992).

[47] K.S. Sandhu, L. Sharma, M. Kaur, R. Kaur, Physical, structural and thermal properties of composite edible films prepared from pearl millet starch and carrageenan gum: process optimization using Response Surface Methodology, Int. J. Biol. Macromol. (2019), https://doi.org/10.1016/j.ijbiomac.2019.09.111.

[48] M.A. Garcia, M.N. Martino, N.E. Zaritzky, Microstructural characterization of plasticized starch-based films, Starch Staerke 52 (2000) 118–124.

[49] M.I.J. Ibrahim, S.M. Sapuan, E.S. Zainudin, M.Y.M. Zuhri, S.M. Sapuan, E.S. Zainudin, M.Y.M. Zuhri, Physical , thermal , morphological , and tensile properties of cornstarch-based films as affected by different plasticizers, Int. J. Food Prop. 22 (2019) 925–941, https://doi.org/10.1080/10942912.2019.1618324.

[50] A. Edhirej, S.M. Sapuan, M. Jawaid, N.I. Zahari, Effect of various plasticizers and concentration on the physical , thermal , mechanical , and structural properties of cassava-starch-based films, Starch - Stärke 68 (2016) 1–11, https://doi.org/10.1002/star.201500366.