UNIVERSITI MALAYSIA PERLIS
International Journal of Nanoelectronics and Materials
IJNeaM
ISSN 1985-5761 | E-ISSN 2232-1535

The optimization of mechanical properties of fish gelatin as biodegradable films

Mohd Shahrulnizam Ahmad [a,c], Roshafima Rasit Ali [b], Aznizam Abu Bakar [c], and Zurina Mohamad [c] *

[a]Radiation Processing Technology Division, Malaysian Institute for Nuclear Technology Research (MINT), Bangi, 43000 Kajang, Selangor, Malaysia
[b]Department of Chemical and Environmental Engineering, Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia, Jalan Sultan Yahya Petra, 54100 Kuala Lumpur, Malaysia
[c]Department of Polymer Engineering, School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor Bahru, Johor, Malaysia
∗Corresponding author. Tel.: +6019-479 1015; e-mail: r-zurina@utm.my

Received 19 August 2024, Revised 13 September 2024, Accepted 25 December 2024

ABSTRACT

The mechanical characteristics of biodegradable polymers are crucial in determining the application of the materials, especially as packaging materials. This work reports the optimization of biodegradable polymer film formulations based on fish gelatin and glycerol, focusing on the tensile strength (Ts) and elongation at break (EB) of biopolymer films. Gelatin extracted from the skin of tilapia fish was dissolved in 100 ml of distilled water and mixed with glycerol as a hydrophilic plasticizer, before being cast and dried to produce films. The content range of fish gelatin and glycerol used is 5.0 to 9.0 g and 10 to 20% (w/w), respectively. This optimization was carried out with central composite design (CCD) using response surface methodology (RSM). The maximum Ts of 27.625 MPa was found at formulation with 9.0 g of gelatin and 10% glycerol, and the optimum EB of 44.578% was recorded at 9.0 g of gelatin with 20% glycerol. These formulations exhibited only 5.12% and 0.60% error between the actual and predicted values. Additionally, Fourier transform infrared spectroscopy (FTIR) also demonstrated the incorporation of glycerol into the fish gelatin film at 20% glycerol, as evidenced by the shifts of Amide A, I, II, and III bands to higher wavenumbers due to hydrogen bonding. This success is also evident from the aliphatic alcohol (C–O molecules derived from glycerol) peaks, observed around 1035 cm^{-1} and 1029 cm^{-1}. These peaks shifted from 1029.99 cm^{-1} to 1035.77 cm^{-1} as the glycerol concentration increased from 10% to 20%. Additionally, the successful incorporation of glycerol resulted in a decrease in intermolecular forces within the films, as evidenced by the Amide A peaks becoming more intense, broader, and sharper.

Keywords: *Morphology, Fish gelatin, Glycerol, Response surface methodology, Tensile strength*

1. INTRODUCTION

Biodegradable materials break down through the enzymatic action of fungi, bacteria, and algae when placed in bioactive environments. Polymer chains can also be disintegrated through non-enzymatic methods, like chemical hydrolysis, to break them down. The metabolism of atmospheric carbon dioxide (CO_2) by plants is a common source of biodegradable materials. They are transformed via biodegradation into CO_2, methane (CH_4), water, biomass, humic matter, and other natural materials [1, 2]. Biodegradable films are essential to the food packaging business since they can replace traditional non-biodegradable plastic films entirely or in part. Biodegradable films can be crafted from edible components like proteins, lipids, and polysaccharides, ensuring their safety for consumption.

These non-biodegradable films also have the potential to seriously harm human life, animal life, and the environment by contributing to environmental contamination [3]. Therefore, the current focus on effectively transforming these biodegradable films into thin, see-through, and adaptable materials arises from their potential use as edible films or coatings for direct contact with food and traditional plastics [4, 5].

Gelatin is a class of proteins that have been extensively researched. It possesses important functional qualities: its capacity to create films and serve as an outer barrier, shielding food from drying out, and exposure to oxygen and light. Gelatin-based films are moisture-sensitive and have low water vapor barrier properties despite having strong mechanical qualities [6]. Mammalian gelatin (bovine and porcine) is the most famous and conventional, but fish gelatin, particularly that from warm-water fish, resembles porcine gelatin and can be used as a substitute for mammalian gelatin in culinary products [7]. The skins and bones of diverse cold-water species like hake, cod, Alaska, salmon, and pollock, as well as warm-water varieties such as catfish, tuna, tilapia, Nile perch, megrim, and shark, have been utilized in the production of fish gelatin.

Plasticizers can reduce the brittle and very easy crack properties of gelatin [8]. A plasticizer's function minimizes gelatin's natural brittleness by lowering intermolecular pressures and increasing polymer-chain mobility and flexibility [9]. Thus, a polymeric system has improved plasticity when a plasticizer has fewer, highly polar groups per molecule and more space between polar groups inside the molecule. Additionally, recent studies have demonstrated that polyols are very effective at plasticizing gelatin films because they enhance intermolecular distance while reducing intermolecular hydrogen bonding. Due to this, numerous studies have concentrated on polyols like sorbitol and glycerol [10, 11].

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques that are beneficial for issue modeling and analysis, to maximize a response that is of interest and affected by several variables [12]. Various researchers used this technique for optimization, such as the development of chitosan-based edible films as food packaging [13], the improvement of gelatin made from chicken skin with various glycerol concentrations [9], and research into some of the physical characteristics of biodegradable films made from mixtures of gelatin and poly (vinyl alcohol) [14]. There are no reports on the mechanical properties optimization for biopolymer films based on glycerol-plasticized fish gelatin. There is interest in studying fish gelatin and glycerol because warm water fish gelatin can replace mammalian gelatin [7] and glycerol is the best plasticizer [10].

This research aimed to produce films utilizing fish gelatin as the primary substance and enhance the mechanical properties of these biopolymer films by incorporating glycerol as a plasticizer. This study primarily examined the effects of three distinct fish gelatin and glycerol content levels on response characteristics crucial for applications in food packaging.

2. MATERIALS AND METHODS

2.1. Materials

Tilapia skin gelatin as warm-water fish with 200 blooms was purchased from Nichz Ingredient Shop, Selangor, Malaysia and distributed by Phywon System Ingredient Sdn Bhd, Selangor. Glycerol was obtained from Biotek Abadi Sdn. Bhd., Selangor, and produced by Chemiz (M) Sdn. Bhd., Selangor.

2.2. Production of Films

Minor adjustments were made to the approach by Nor *et al.* [15] and Krishna *et al.* [16]. Response Surface Methodology (RSM) predictive formulations were used to create 13 filmogenic solutions, as shown in Table 1.

Using a mechanical stirrer at room temperature, quantities of fish gelatin powder weighing 5.0, 7.0, and 9.0 g were mixed in distilled water with a capacity of 100 ml for each. Respective portions of glycerol from 10, 15, and 20% (w/w) were then added to the solution of fish gelatin. All mixtures were subjected to heat at 50 °C for 10 minutes under continuous stirring to obtain homogenous solutions. Each filmogenic solution was added to a clean Petri dish in a quantity of around 25 ml, and afterwards, the plates were subjected to a drying process for 48 hours at 45 °C. Then, dried films were peeled off to undergo tensile strength and elongation at break testing.

2.3. RSM Optimisation of Fish Gelatin-Glycerol Films

The optimized formulation was suggested using response surface methodology (RSM) to produce fish gelatin and glycerol films. The factors or independent variables involved various glycerol concentrations and quantities of fish gelatin in optimizing the responses: tensile strength and elongation at break, as depicted in Table 2. The central composite design (CCD) process randomly selected 13 film formulas for the design (Table 1). For (A) fish gelatin at quantities 5.0, 7.0, and 9.0 g and (B) glycerol contents at 10, 15, and 20% (w/w), two manipulated variables were applied at three equidistant levels (−1, 0, +1).

2.4. Determination of Tensile Strength and Elongation at Break (EB)

Ts and EB values were determined and operated by a Universal Tensile Machine 20 kN (Shimadzu AG-Xplus) from Kyoto, Japan, with the ASTM D882 method. Filmstrips measuring 60 mm × 15 mm and a thickness range of 0.20 to 0.30 mm, were get ready with a cutting blade. The gauge length was 40 mm, and filmstrips were elongated with 10 mm/min speed until fracture. Ts (N/mm^2) was calculated as Equation (1):

Table 1. Fish gelatin film codes, as well as the conditions and outcomes of 13 trial runs

Runs	Factors		Responses	
	Fish gelatin contents (g)	Glycerol contents (% w/w)	Tensile strength (N/mm^2)	Elongation at break (%)
1	7	20	6.51	38.70
2	7	15	8.63	21.92
3	5	20	10.23	24.56
4	9	10	29.04	2.83
5	7	15	11.78	28.83
6	7	15	12.19	32.54
7	5	10	21.57	11.43
8	9	15	13.66	31.98
9	7	15	15.23	39.39
10	7	15	9.08	22.72
11	5	15	14.24	25.27
12	9	20	10.60	44.31
13	7	10	19.68	7.07

Table 2. Variables involved in this study

Independent variables (Numerical Factors)	Dependent variables (Responses)
Fish gelatin contents (g)	Tensile strength (N/mm^2) (6.51–29.04)
Glycerol contents (% w/w)	Elongation at break (%) (2.83–44.31)

$$\text{Tensile Strength} = \frac{F_{\max}\,(N)}{A\,(m^2)} \tag{1}$$

where $F_{\max}$ is the optimum load (N) necessary for fracture and (A) is the cross-sectional area (m^2) of the film samples.

The percentage of EB was calculated using Equation (2):

$$\text{Elongation at Break} = \frac{I_{\max}}{I_0} \times 100 \tag{2}$$

$I_{\max}$ is film elongation (mm) at fracture, and I_0 is the sample's starting grip length (mm).

2.5. Fourier Transform Infrared Spectroscopy (FTIR) Interpretation

Intermolecular crosslinking of biopolymers was identified using FTIR, which was also used to track modifications to the functional group and secondary structure. With some adjustments of suitable parameters, FTIR was used to identify the sample structure. The sample for each formulation was cut into 1 cm^2 pieces and measured at a distance of 4 cm^{-1} for 16 scans at a wavelength between 4000–400 nm using IRTracer-100 Shimadzu from Kyoto, Japan [15]. The comparison amounts of glycerol were used to observe the differences in spectra caused by the increase in glycerol concentrations.

3. RESULTS AND DISCUSSION

For all packing applications, film packaging materials must preserve integrity and barrier qualities while keeping sufficient mechanical strength and elasticity to withstand external force. Table 1 demonstrates that adding glycerol reduces the Ts of biopolymer films. This is a result of glycerol plasticization, which increased flexibility and stretchability by weakening the connections between the biopolymer components of the protein film [9, 15]. For example, it was possible to see that the Ts reduced from 21.57, 14.24, and 10.23 N/mm^2 at 10%, 15%, and 20% of glycerol, respectively, at 5 g of gelatin/100 ml distilled water. It may also be demonstrated that the effects of glycerol cause Ts of 7 and 9 g of gelatin to drop. Moreover, the glycerol proportion was raised, and the high plasticizing action of glycerol decreased tensile strength [17, 18]. The molecular weight of the plasticizer was used to explain this behavior, where the free volume and disruption of the molecular network increased. As a result, the number of plasticizer molecules in a specific film can be treated as a function of the film's qualities.

EB is also called "fracture strain", the ratio between the examined sample's modified length and its beginning length upon rupture. EB is a crucial characteristic of food packaging films since it exhibits a film's capacity to withstand shape alters without developing cracks [9]. Table 1 showed that EB enhanced while tensile strength declined with rising glycerol concentrations. The plasticizing effect of glycerol, which reduces connections between neighboring chains in the biopolymer matrix and increases movability and film flexibility, is most likely the cause of this activity. Among the examples of plasticizers used in some studies of films are glycerol [8, 17], sorbitol and mannitol [19, 20] and lignin [21]. In addition, the smaller molecular structure of glycerol is responsible for this because it reduces intermolecular pressures along the chains of polymer molecules, improving film flexibility even as barrier characteristics decline [19, 22].

While this happened, the mechanism factors' direct effects on the relationship and response effects were resolved using ANOVA and 3D surface plots. This allowed for the computation of the concurrent effects of numerous variables on the Ts and EB of the plasticized fish gelatin film, which is covered in depth in this section. After that, based on the changes in the two variables, the excellent pairing of the two process variables was approximated.

3.1. Analysis of Ts and EB Variance (ANOVA)

The ANOVA values for Ts and EB were displayed in Tables 3 and 4, respectively. The F-value, the ratio of mean square regression to the value of residual, was used to assess the significance of the created model. Low error magnitudes are correlated with high F-values [23]. Ts and EB analyses demonstrated an F-value of 14.90 and 10.75, respectively. However, the significance for both models can be proved from the *P*-value; 0.13% for Ts and 0.35% for EB. These values presented only a slight chance that an *F*-value could occur due to noise. The principal parameters of the prediction model are determined by this P-value or probability value, and their relatedness effects are significant (must be less than 0.05). Both models also could see that variable B is substantial to the impacts of Ts and EB. Otherwise, this variable B is also essential along with the interaction effect A^2 and AB for Ts and EB, respectively.

Furthermore, both Ts and EB models implied irrelevant ratios of lack of fit to the pure error, which are 0.5178 and 0.0524, respectively. The *p*-value for Ts is calculated at 69.22%, and for EB is 98.12%. Thus, this indicates that both models adequately fit the data.

In a regression model, R-squared (or R^2), also termed the coefficient of determination, serves as a statistical metric indicating the proportion of variability in the dependent variable explained by the independent variable. This R^2 is very important to be considered because it shows how well the data is adequate for the regression model. This part of the analysis was done by appraising R^2, adjusted R^2, and predicted R^2. Nor *et al.* [23] stated that greater R-squared values indicate the model's adequacy and the accuracy of the estimated coefficients. Predicted R^2 can be more helpful for model comparison than adjusted R^2 because it is derived from observations not considered during model estimation. To be in reasonable agreement, the predicted R^2 and adjusted R^2 coefficients, which reflect how well the polynomial fits, should be within about 0.20 of one another [24]. From Table 3, the analysis for Ts in this study showed an R^2 of 0.9141, which indicates that the model could accommodate 91.41% of the variability and adequately correspond with the experimental data. Accordingly, the predicted R^2 and adjusted R^2 are in a good deal (the difference is less than 0.20), suggesting that the experimental and predicted efficiencies, with values of 0.8528 and 0.6894, respectively, are not significantly different. Moreover, the R^2, adjusted $R^{2,}$ and predicted R^2 for the EB analysis are 0.8848, 0.8024, and 0.7958, respectively (Table 4).

To choose the best-fitting model, regression analysis was also performed for several models, including linear, quadratic, and 2FI. Simultaneously, for Ts and EB in Equations 3 and 4, respectively, successive ANOVA defined the entire predictive model as a quadratic polynomial model.

$$\mathrm{Ts} = 79.09466 - 9.43267\mathrm{A} - 3.51751\mathrm{B} - 0.177500\mathrm{AB} + 0.907155\mathrm{A}^2 + 0.110945\mathrm{B}^2 \quad (3)$$

$$\mathrm{EB} = -28.04217 - 4.76671\mathrm{A} + 6.30183\mathrm{B} + 0.70875\mathrm{AB} - 0.312586\mathrm{A}^2 - 0.279614\mathrm{B}^2 \quad (4)$$

Moreover, Figure 1 displays a straight line generated by the normal probability plot of the residuals for the Ts and EB, indicating that the errors are regularly distributed and that an appropriate model has been created. An acceptable model was suggested by the residual points' distribution around the straight line on the normal % probability against internally studentized residual plots. Regression analysis was used to resolve if the model adequately captured the experimental data by examining residual behaviors.

Another tool for selecting a suitable model is the dispersion of data points around the response variable's mean. A data point that is evenly dispersed around the mean of the response variable suggests that the model is adequate for the data (Figure 2). The relationship between the actual current values from the experiment and expected response values based on the quadratic model equation was evaluated using a predicted vs actual plot. This also indicates that an excellent relationship to the linear regression fit is obtained in this graph, showing that the model sufficiently explains the experimental data, with R^2 values of 0.9141 for Ts and 0.8848 for EB.

Plots of the final ANOVA, Ts, and EB residuals against predicted values are displayed in Figure 3. The figure indicates that there were no apparent scattering residuals for either the tensile strength or elongation at break responses. Thus, the proposed model was suitable, and there was no proof that the constant ANOVA assumption or accuracy had been invalidated.

Additionally, Table 5 compares the measured and matched predicted data. Ts and EB's maximum errors were 37.45% and 34.39%, respectively. Equations (5) and (6) are used to calculate this error:

$$\text{Residual} = \text{Actual value} - \text{Predicted value} \quad (5)$$

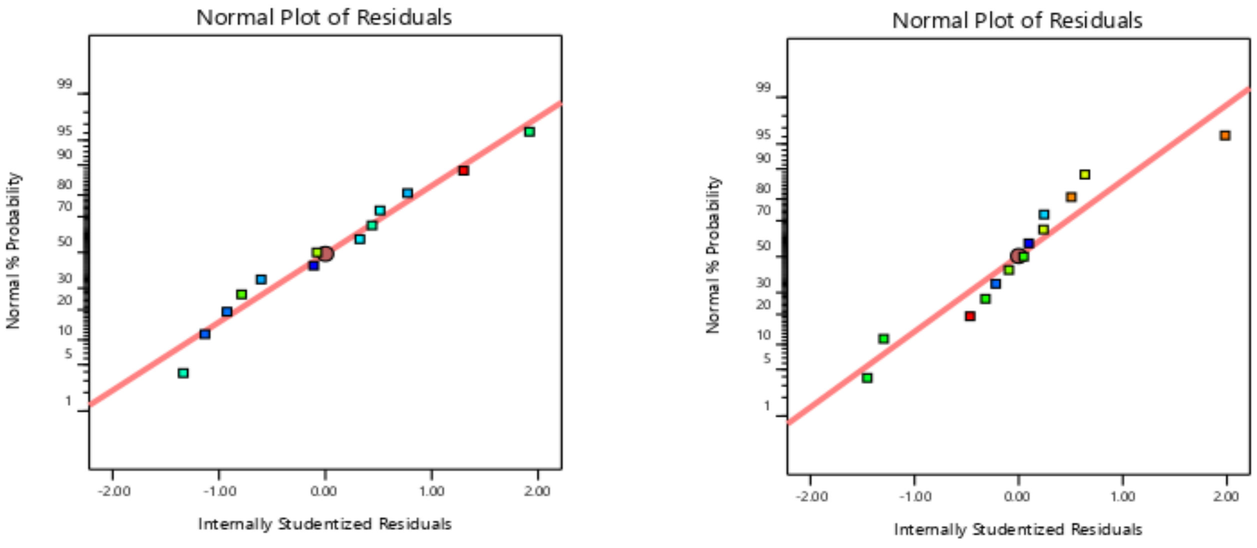
$$\text{Residual Error}(\%) = \frac{|\text{Actual value} - \text{Predicted value}|}{\text{Predicted value}} \times 100\% \quad (6)$$

Table 3. ANOVA analysis for tensile strength

Source	Sum of squares	df	Mean square	*F*-value	*P*-value	
Model	421.00	5	84.20	14.90	0.0013	**Significant**
A – Fish gelatin contents	8.78	1	8.78	1.55	0.2526	
B – Glycerol contents	307.45	1	307.45	54.41	0.0002	
AB	12.60	1	12.60	2.23	0.1790	
A^2	36.37	1	36.37	6.44	0.0388	
B^2	21.25	1	21.25	3.76	0.0937	
Residual	39.56	7	5.65			
Lack of fit	11.06	3	3.69	0.5178	0.6922	**Not Significant**
Pure error	28.49	4	7.12			
Cor Total	460.56	12				
		R^2 = 0.9141		Adjusted R^2 = 0.8528		Predicted R^2 = 0.6894

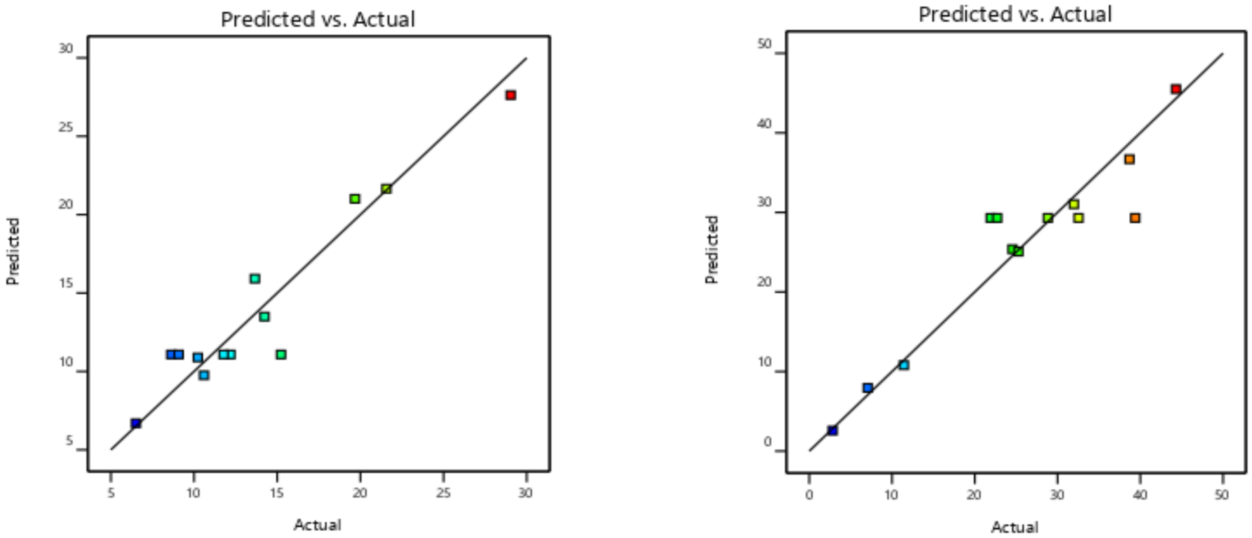
Table 4. ANOVA analysis for elongation at break

Source	Sum of squares	df	Mean square	*F*-value	*P*-value	
Model	1678.09	5	335.62	10.75	0.0035	**Significant**
A – Fish gelatin contents	53.16	1	53.16	1.70	0.2332	
B – Glycerol contents	1239.56	1	1239.56	39.70	0.0004	
AB	200.93	1	200.93	6.43	0.0389	
A^2	4.32	1	4.32	0.1383	0.7210	
B^2	134.96	1	134.96	4.32	0.0762	
Residual	218.58	7	31.23			
Lack of fit	8.54	3	2.85	0.0542	0.9812	**Not Significant**
Pure error	210.05	4	52.51			
Cor Total	1896.67	12				
		R^2 = 0.8848		Adjusted R^2 = 0.8024		Predicted R^2 = 0.7958



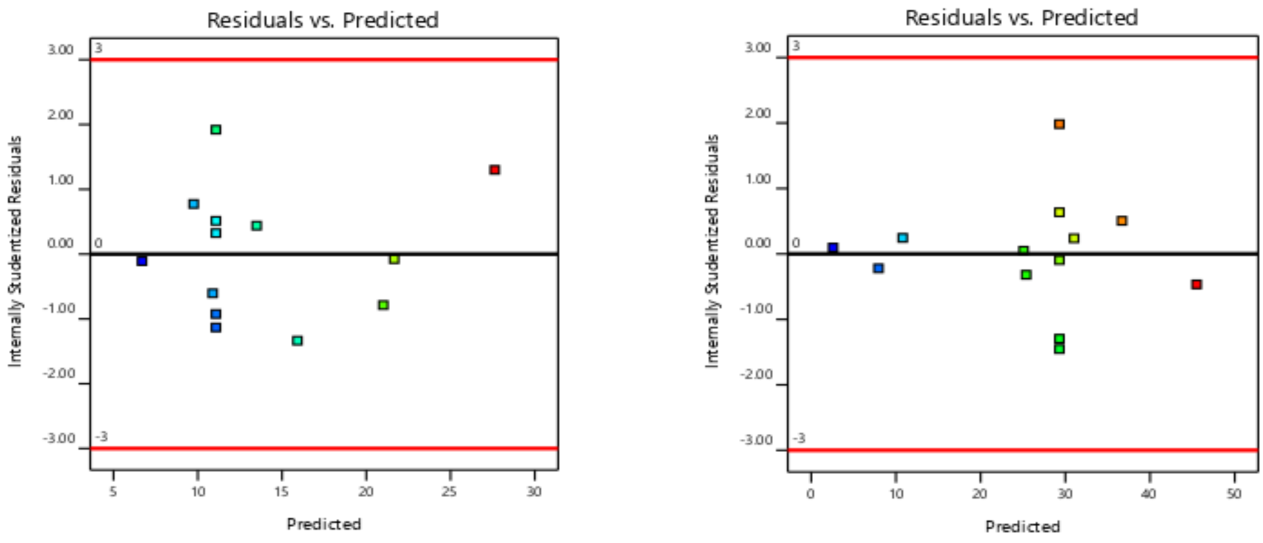
(a) (b)

Figure 1. Normal plot of residuals for (a) tensile strength and (b) elongation at break



(a) (b)

Figure 2. Predicted versus actual values for (a) tensile strength and (b) elongation at break



(a) (b)

Figure 3. Internally studentized residuals versus predicted values for (a) tensile strength and (b) elongation at break

Table 5. Actual value, predicted value, residual, and error for the Ts and EB

Run	Tensile strength (N/mm^2)				Elongation at break (%)			
	Actual	Predicted	Residual	Error (%)	Actual	Predicted	Residual	Error (%)
1	6.51	6.69	−0.18	2.69	38.70	36.69	2.01	5.48
2	8.63	11.08	−2.45	22.11	21.92	29.31	−7.39	25.21
3	10.23	10.89	−0.66	6.06	24.56	25.38	−0.82	3.23
4	29.04	27.62	1.42	5.14	2.83	2.58	0.25	9.69
5	11.78	11.08	0.70	6.32	28.83	29.31	−0.48	1.64
6	12.19	11.08	1.11	10.02	32.54	29.31	3.23	11.02
7	21.57	21.65	−0.08	0.37	11.43	10.80	0.63	5.83
8	13.66	15.92	−2.26	14.20	31.98	31.03	0.95	3.06
9	15.23	11.08	4.15	37.45	39.39	29.31	10.08	34.39
10	9.08	11.08	−2.00	18.05	22.72	29.31	−6.59	22.48
11	14.24	13.50	0.74	5.48	25.27	25.08	0.19	0.76
12	10.60	9.76	0.84	8.61	44.31	45.50	−1.19	2.62
13	19.68	12.01	−1.33	6.33	7.07	7.94	−0.87	10.96

3.2. 3D Surface Plots to Show How Processing Variables Affect Ts and EB

3.2.1. The Effects of Fish Gelatin and Glycerol Contents (A-B Interaction)

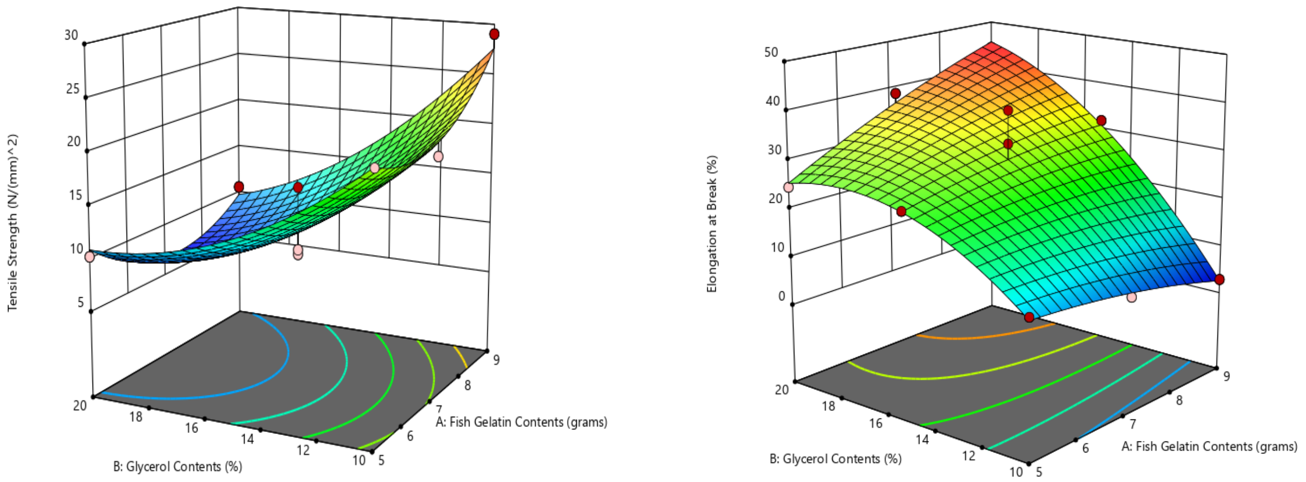
Fish gelatin and glycerol contents interacted with one another to affect Ts and EB, as shown by the 3D response surface plots in Figure 4. The figure shows that the Ts decreased and EB increased significantly with glycerol. This can be proved by the study on the impact of the physical characteristics of gelatin plasticized with glycerol and sorbitol mixtures [18] and the effect of sorbitol at various concentrations on films made of bovine gelatin [25]. These glycerol and sorbitol are believed to act as a plasticizer that might minimize protein-protein interaction and increase the mobility of polypeptide chains in the protein structure. Additionally, through branching/grafting circumstances, plasticizing effects at high concentrations affect the expansion of free volume inside the film matrix and molecular mobility [26].

Otherwise, it is advised to integrate plasticizers with protein film to improve the flexibility of the film matrix. Plasticizers are low molecular weight molecules [6]. Most protein-based films have an impact but are brittle without plasticizers [15]. Therefore, plasticizers are required to increase the potential for the application of protein-based films. Additionally, plasticizers are thought to improve protein film elongation and flexibility while influencing permeability. Plasticizer also affects the film's solubility as well. The appearance and elongation of protein films would be enhanced by increasing their water solubility [27].

Additionally, glycerol is more hygroscopic and has a smaller molecular weight than other plasticizers and glycerol is the limited molecular weight of plasticizers [10, 27]. This contributes to the fact that glycerol is a better plasticizer that can increase the polymer matrix's mobility, elasticity, and flexibility.

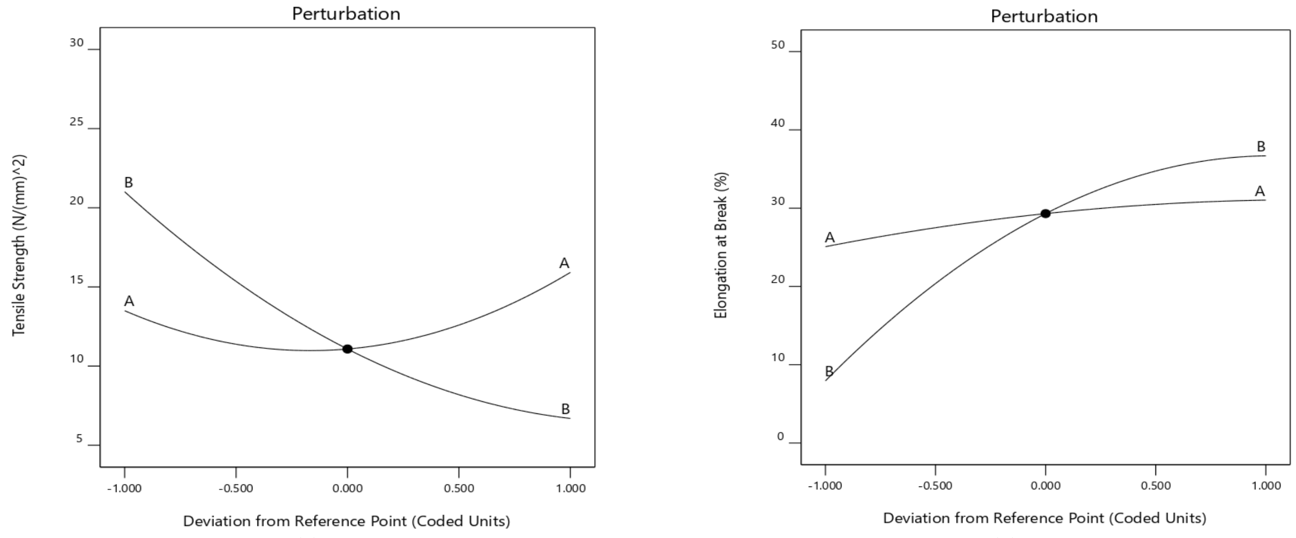
3.3. Main Effect Plot

Figure 5 explains the influences of the fish gelatin and glycerol contents on the mechanical characteristics of the hybrid biocomposites. The graphs demonstrate that the glycerol content significantly influences both Ts and EB when considering all two independent variables.



(a) (b)

Figure 4. The impact of varying fish gelatin and glycerol levels is depicted in 3D surface plots for both (a) tensile strength and (b) elongation at break



(a) (b)

Figure 5. The main effects plot for (a) tensile strength; (b) elongation at break

Gelatin's Bloom value is expressed as gel strength, a gauge of the material's stiffness and strength [4]. Hence, the stiffness properties of gelatin can induce its brittleness, which can be seen from the perturbation plots. Increasing the amount of glycerol (B) is very significant in the decline in Ts and increase in EB (Figures 5 (a) and 5 (b)). The perturbation plots explained the comparison effect of every factor [28]. By lowering intermolecular stress, the plasticizing chemicals have assisted in lessening the natural brittleness of gelatin films [8]. Many works have been successfully reported on the addition of glycerol as a plasticizer in gelatin films [15, 16, 18, 29].

The successful incorporation of glycerol in fish gelatin films can be shown from FTIR Spectroscopy studies, as depicted in Figure 6. The studies showed six different peaks marked as Amide A, which was observed to be connected to stretching vibrations of N–H between 3500 to 3100 cm⁻¹. Amide I is a representation of hydrogen bonding and C=O stretching combined with COO; Amide II is the product of N–H groups' bending and stretching vibrations; and the vibrations of the C–N and N–H groups of bound amides are associated with Amide III [30].

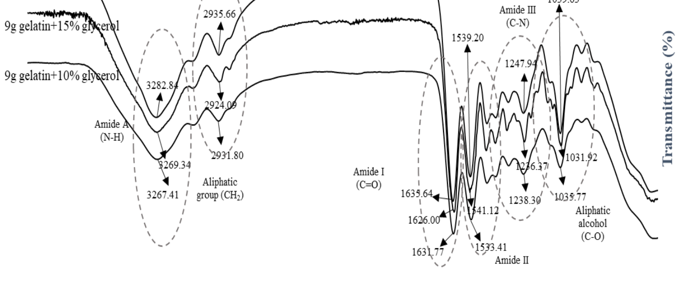


Figure 6. FTIR spectra of fish gelatin films with glycerol incorporation

The peaks around 1039 and 1031 cm⁻¹ showed the presence of aliphatic alcohol, which is composed of C–O molecules derived from glycerol. The peak from 1035.77 cm⁻¹ (10% glycerol) increased to 1039.63 cm⁻¹ (20% glycerol), indicating that the peak wavenumber increased as the glycerol concentration increased. Moreover, further interactions between the plasticizer and the film structure have contributed to the displacement [31]. As the peak of aliphatic alcohol shifted to a higher wavenumber as glycerol content increased, it also affected the N–H bands from 3267.41 to 3282.84 cm⁻¹ (Amide A). This peak became more intense, wider, and sharper as the amount of glycerol in the matrix films increased, perhaps due to O–H molecules supplied by the plasticizer [15, 32]. In contrast, the peak of amide A became more intense and sharper when glycerol was incorporated up to 40% in pigskin gelatin films.

From 1650 cm⁻¹ to 1230 cm⁻¹, the peaks of Amides I, II and III, respectively, were seen. As the glycerol concentration increased, the intensity and wavenumber of these peaks also increased. This is primarily due to bands of C=O, N–H, and C–N in protein-film structures having the propensity to create hydrogen bonds with the O–H from glycerol intermolecularly [15]. The peaks' intensities of Amide I and Amide II increased by adding glycerol and iodine at 30% and 8% of contents, respectively [33].

The aliphatic group (CH_2) peak at 2930 to 2920 cm⁻¹ was observed. The wavenumber slightly shifts to a higher value. This is probably due to the higher glycerol concentration, whereas the hydrogen bond formed in protein-film structure with O–H molecules from glycerol. In contrast, the peak of this aliphatic group shifted to a lower value by adding palm oil. This is caused by the dilution effect of palm oil in the protein-film structure network [30].

3.4. Optimization of the Response

In this phase, tensile strength (Ts) and elongation at break (EB) were two mechanical characteristics optimized using the Design-Expert Software's response optimizer. From

Table 6, the optimum values for Ts and EB obtained are 27.625 N/mm^2 and 44.578%, respectively, with the desirability of 0.937 and 1.000. The independent variables yielding this highest response are 9 g of gelatin, 10% glycerol for Ts, and 8.938 g, 19.563% for EB. Otherwise, the data also shows that percentage errors for Ts and EB are 5.122% and 0.601% for Ts and EB, respectively. Maximizing tensile strength and elongation at break was the objective; therefore, the software can evaluate the maximum value to compare its differences with the experimental value.

Table 6. Validation results for optimized fish gelatin and glycerol as biodegradable films

Parameters	A (g)	B (% w/w)	Ts (N/mm^2)	Elongation at break (%)	Desirability
Actual for Ts	9.000	10.000	29.040		
Actual for EB	9.000	20.000		44.310	
Predicted for Ts	9.000	10.000	27.625		0.937
Predicted for EB	8.938	19.563		44.578	1.000
Error (%) for Ts	0.000		5.122		
Error (%) for EB		2.234		0.601	

4. CONCLUSION

The fish gelatin-based film with the glycerol has been successfully developed in this research. The brittleness and tendency of gelatin films to crack were reduced by adding glycerol. The result of optimized formulation by RSM showed that the 9.0 g with 10% glycerol for tensile strength (Ts) and 9.0 g with 20% glycerol for elongation at break (EB). The RSM analysis also suggested that the highest desirability of predicted value and percentage error from actual value is only 5.122% (Ts) and 0.601% (EB). FTIR studies confirmed the addition of glycerol in protein-film network structure. The peaks of Amides A, I, II and III and even aliphatic groups increased by the wavenumber and intensities as glycerol content increased. This suggests successful formation of intermolecular bonds between O–H molecules and N–H, C=O, C–N, and CH_2.

ACKNOWLEDGMENTS

This study was financed by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS) "Synergistic plasticization and cross-linking effect on the properties of warm water fish gelatin-based biopolymer films" (FRGS/1/2023/TK09/UTM/02/19).

REFERENCES

[1] A. Folino, A. Karageorgiou, P. S. Calabrò, and D. Komilis, “Biodegradation of Wasted Bioplastics in Natural and Industrial Environments: A Review,” *Sustainability*, vol. 12, no. 15, p. 6030, Jul. 2020, doi: 10.3390/su12156030.

[2] R. A. Gross and B. Kalra, “Biodegradable Polymers for the Environment,” *Science (1979)*, vol. 297, no. 5582, pp. 803–807, Aug. 2002, doi: 10.1126/science.297.5582.803.

[3] A. Siti Amirah and K. I. Ku Marsilla, “Effect of Glycerol Compositions on Protein Based Thermoplastic from Fish Waste,” *Journal of Physics: Conference Series*, vol. 1082, p. 012012, Aug. 2018, doi: 10.1088/1742-6596/1082/1/012012.

[4] Z. A. Nur Hanani, Y. H. Roos, and J. P. Kerry, “Use and application of gelatin as potential biodegradable packaging materials for food products,” *International Journal of Biological Macromolecules*, vol. 71, pp. 94–102, Nov. 2014, doi: 10.1016/j.ijbiomac.2014.04.027.

[5] S. Kandasamy *et al.*, “Application of Whey Protein-Based Edible Films and Coatings in Food Industries: An Updated Overview,” *Coatings*, vol. 11, no. 9, p. 1056, Aug. 2021, doi: 10.3390/coatings11091056.

[6] N. Suderman, M. I. N. Isa, and N. M. Sarbon, “The effect of plasticizers on the functional properties of biodegradable gelatin-based film: A review,” *Food Bioscience*, vol. 24, pp. 111–119, Aug. 2018, doi: 10.1016/j.fbio.2018.06.006.

[7] J. Park, J. Nam, H. Yun, H.-J. Jin, and H. W. Kwak, “Aquatic polymer-based edible films of fish gelatin crosslinked with alginate dialdehyde having enhanced physicochemical properties,” *Carbohydrate Polymers*, vol. 254, p. 117317, Feb. 2021, doi: 10.1016/j.carbpol.2020.117317.

[8] M. G. A. Vieira, M. A. da Silva, L. O. dos Santos, and M. M. Beppu, “Natural-based plasticizers and biopolymer films: A review,” *European Polymer Journal*, vol. 47, no. 3, pp. 254–263, Mar. 2011, doi: 10.1016/j.eurpolymj.2010.12.011.

[9] N. Suderman and N. M. Sarbon, “Optimization of chicken skin gelatin film production with different glycerol concentrations by response surface methodology (RSM) approach,” *Journal of Food Science and Technology*, vol. 57, no. 2, pp. 463–472, Feb. 2020, doi: 10.1007/s13197-019-04074-0.

[10] A. A. Al-Hassan and M. H. Norziah, “Starch–gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers,” *Food Hydrocolloids*, vol. 26, no. 1, pp. 108–117, Jan. 2012, doi: 10.1016/j.foodhyd.2011.04.015.

[11] A. Sancakli, B. Basaran, F. Arican, and O. Polat, “Effects of bovine gelatin viscosity on gelatin-based edible film mechanical, physical and morphological properties,” *SN Applied Sciences*, vol. 3, no. 1, p. 8, Jan. 2021, doi: 10.1007/s42452-020-04076-0.

[12] M. Y. Noordin, V. C. Venkatesh, S. Sharif, S. Elting, and A. Abdullah, “Application of response surface methodology in describing the performance of coated carbide tools when turning AISI 1045 steel,” *Journal of Materials Processing Technology*, vol. 145, no. 1, pp. 46–58, Jan. 2004, doi: 10.1016/S0924-0136(03)00861-6.

[13] T. P. Singh, M. K. Chatli, and J. Sahoo, "Development of chitosan based edible films: process optimization using response surface methodology," *Journal of Food Science and Technology*, vol. 52, no. 5, pp. 2530–2543, May 2015, doi: 10.1007/s13197-014-1318-6.

[14] R. A. Carvalho *et al.*, "Study of some physical properties of biodegradable films based on blends of gelatin and poly(vinyl alcohol) using a response-surface methodology," *Materials Science and Engineering: C*, vol. 29, no. 2, pp. 485–491, Mar. 2009, doi: 10.1016/j.msec.2008.08.030.

[15] M. H. M. Nor, N. N. M. Nazmi, and N. M. Sarbon, "Effects of plasticizer concentrations on functional properties of chicken skin gelatin films," *International Food Research Journal*, vol. 24, no. 5, pp. 1910 – 1918, 2017.

[16] M. Krishna, C. I. Nindo, and S. C. Min, "Development of fish gelatin edible films using extrusion and compression molding," *Journal of Food Engineering*, vol. 108, no. 2, pp. 337–344, Jan. 2012, doi: 10.1016/j.jfoodeng.2011.08.002.

[17] S. Faust, J. Foerster, M. Lindner, and M. Schmid, "Effect of glycerol and sorbitol on the mechanical and barrier properties of films based on pea protein isolate produced by high-moisture extrusion processing," *Polymer Engineering & Science*, vol. 62, no. 1, pp. 95–102, Jan. 2022, doi: 10.1002/pen.25836.

[18] M. Thomazine, R. A. Carvalho, and P. J. A. Sobral, "Physical Properties of Gelatin Films Plasticized by Blends of Glycerol and Sorbitol," *Journal of Food Science*, vol. 70, no. 3, pp. E172–E176, May 2006, doi: 10.1111/j.1365-2621.2005.tb07132.x.

[19] L. Ballesteros-Mártinez, C. Pérez-Cervera, and R. Andrade-Pizarro, "Effect of glycerol and sorbitol concentrations on mechanical, optical, and barrier properties of sweet potato starch film," *NFS Journal*, vol. 20, pp. 1–9, Aug. 2020, doi: 10.1016/j.nfs.2020.06.002.

[20] N. Cao, X. Yang, and Y. Fu, "Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films," *Food Hydrocolloids*, vol. 23, no. 3, pp. 729–735, May 2009, doi: 10.1016/j.foodhyd.2008.07.017.

[21] R. Núñez-Flores, B. Giménez, F. Fernández-Martín, M. E. López-Caballero, M. P. Montero, and M. C. Gómez-Guillén, "Physical and functional characterization of active fish gelatin films incorporated with lignin," *Food Hydrocolloids*, vol. 30, no. 1, pp. 163–172, Jan. 2013, doi: 10.1016/j.foodhyd.2012.05.017.

[22] F. M. Vanin, P. J. A. Sobral, F. C. Menegalli, R. A. Carvalho, and A. M. Q. B. Habitante, "Effects of plasticizers and their concentrations on thermal and functional properties of gelatin-based films," *Food Hydrocolloids*, vol. 19, no. 5, pp. 899–907, Sep. 2005, doi: 10.1016/j.foodhyd.2004.12.003.

[23] A. F. M. Nor, M. Z. Hassan, Z. A. Rasid, S. A. Aziz, S. Sarip, and M. Y. Md Daud, "Optimization on Tensile Properties of Kenaf/Multi-walled CNT Hybrid Composites with Box-Behnken Design," *Applied Composite Materials*, vol. 28, no. 3, pp. 607–632, Jun. 2021, doi: 10.1007/s10443-021-09879-x.

[24] H. Yaghoobi and A. Fereidoon, "Modeling and optimization of tensile strength and modulus of polypropylene/kenaf fiber biocomposites using Box–Behnken response surface method," *Polymer Composites*, vol. 39, no. S1, Apr. 2018, doi: 10.1002/pc.24596.

[25] N. F. Bakry, M. I. N. Isa, and N. M. Sarbon, "Effect of sorbitol at different concentrations on the functional properties of gelatin/carboxymethyl cellulose (CMC)/chitosan composite films," *International Food Research Journal*, vol. 24, no. 4, pp. 1753 – 1762, 2017.

[26] I. Choi, S. E. Lee, Y. Chang, M. Lacroix, and J. Han, "Effect of oxidized phenolic compounds on cross-linking and properties of biodegradable active packaging film composed of turmeric and gelatin," *LWT*, vol. 93, pp. 427–433, Jul. 2018, doi: 10.1016/j.lwt.2018.03.065.

[27] K. Dangaran, P. M. Tomasula, and P. Qi, "Structure and Function of Protein-Based Edible Films and Coatings," in *Edible Films and Coatings for Food Applications*, New York, NY: Springer New York, 2009, pp. 25–56. doi: 10.1007/978-0-387-92824-1_2.

[28] R. Srinivasan, T. Pridhar, L. S. Ramprasath, N. S. Charan, and W. Ruban, "Prediction of tensile strength in FDM printed ABS parts using response surface methodology (RSM)," *Materials Today: Proceedings*, vol. 27, pp. 1827–1832, 2020, doi: 10.1016/j.matpr.2020.03.788.

[29] Z. A. Nur Hanani, Y. H. Roos, and J. P. Kerry, "Use of beef, pork and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties," *Food Hydrocolloids*, vol. 29, no. 1, pp. 144–151, Oct. 2012, doi: 10.1016/j.foodhyd.2012.01.015.

[30] K. Nilsuwan, S. Benjakul, and T. Prodpran, "Influence of palm oil and glycerol on properties of fish skin gelatin-based films," *Journal of Food Science and Technology*, vol. 53, no. 6, pp. 2715–2724, Jun. 2016, doi: 10.1007/s13197-016-2243-7.

[31] Z. A. Nur Hanani, J. McNamara, Y. H. Roos, and J. P. Kerry, "Effect of plasticizer content on the functional properties of extruded gelatin-based composite films," *Food Hydrocolloids*, vol. 31, no. 2, pp. 264–269, Jun. 2013, doi: 10.1016/j.foodhyd.2012.10.009.

[32] P. Bergo and P. J. A. Sobral, "Effects of plasticizer on physical properties of pigskin gelatin films," *Food Hydrocolloids*, vol. 21, no. 8, pp. 1285–1289, Dec. 2007, doi: 10.1016/j.foodhyd.2006.09.014.

[33] N. Singh, D. M. R. Georget, P. S. Belton, and S. A. Barker, "Zein–Iodine Complex Studied by FTIR Spectroscopy and Dielectric and Dynamic Rheometry in Films and Precipitates," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 10, pp. 4334–4341, May 2009, doi: 10.1021/jf900436q.