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Article

The effects of plasticizers on the mechanical properties and chemical composition of a gelatin biopolymer

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SUMMARY

Every day, oil-based plastics are manufactured, consumed, and discarded without a second thought. To combat plastic pollution, we decided to investigate gelatin bioplastics. We analysed the effects of the increasing concentration of polyethylene glycol (PEG) and glycerol plasticizers on the mechanical properties and chemical composition of the gelatin bioplastic matrix through a series of experiments. In the first experiment, we determined their tensile strength (TS) and the elongation at break (EAB) through tensile testing. We hypothesised that increasing concentrations of the plasticizers would decrease TS and increase EAB. In the second experiment, we studied their O–H bonds by Fourier-transform infrared spectroscopy analysis (FTIR). Our second hypothesis maintained that the number of O–H bonds would increase because plasticizers disrupt polymer-polymer and polymer-water hydrogen bonds. For the mechanical properties, we observed that when the concentration of PEG and glycerol plasticizers increased so too did EAB. The rising concentrations of glycerol led to a decreasing trend with TS, whereas PEG showed an increasing trend. The FTIR spectrum revealed that a high abundance of O–H bonds was present at the strong and broad absorption peak of 3400 cm-1, with the pure gelatin films having the highest absorption, followed by glycerol-plasticized films and PEG-plasticized films having the least absorption. We concluded that 3% w/v PEG film outperformed other PEG films and 3% w/v glycerol films. The cytotoxicity results showed that all films had a cell viability above the threshold of 75% and hence food-safe.

INTRODUCTION

Over 12 million tonnes of oil-based plastic are dumped into the ocean yearly, accounting for 18.5% of all terrestrial municipal solid waste (1, 2). Oil-based plastics are made from fossil fuel derivatives which bring forth devastating consequences on the environment, as microplastics propagate throughout the food chain (3, 4). The inert nature and high crystallinity of these oil-based plastics mean it may take more than 50 years for them to degrade (5). Bioplastics are a promising alternative to conventional plastics. They are biodegradable, renewable, economically feasible, and easily implementable. Gelatin shows potential as a viable starting point.

Gelatin is a polypeptide molecule derived from the partial hydrolysis of collagen, an abundant animal protein that constitutes approximately 30% of the total protein mass in mammals (6). Collagen has a stable triple-helix crystalline conformation that gives it superior mechanical properties in supporting tendons and muscle groups (7). As gelatin can be extracted from meat byproducts, gelatin bioplastics are not only biodegradable but contribute to an external circular economy. Biodegradable plasticizers are added to enhance flexibility and durability (8). Glycerol is one option; it has a high compatibility with gelatin and a high thermal capacity (9). It is edible and food-safe. Likewise, PEG is a suitable plasticizer as it is biodegradable and soluble in water (10).

This investigation aimed to compare the physical properties and chemical composition of gelatin biofilms in this work, plasticized with either PEG or glycerol. Although previous studies have been conducted to show the independent properties of each plasticizer, rarely do they compare plasticizers to determine which is more suitable for our daily lives. In our first experiment, we measured the TS and EAB using tensile testing. Tensile stress is the force per unit cross-sectional area exerted on a material when it is pulled (11). TS is the maximum stress that the material can withstand before fracture (11). Tensile strain is the percentage elongation of the material as it is pulled (11). The EAB is the percentage elongation just before fracture. It is a measure of ductility (11). We hypothesised that increases in the concentration of each plasticizer would decrease TS and increase EAB. On a molecular level, PEG is an external plasticizer (12). It cannot chemically attach to the polymer backbone. This unstable connection helps to facilitate gelatin-chain mobility. Conversely, glycerol acts as an internal plasticizer (13). Hydrogen bonds are formed between the hydroxyl groups of gelatin chains and glycerol. It disrupts the gelatin-water and gelatin-gelatin bonds in the matrix (14). By conducting FTIR analysis in the second experiment, we investigated the O–H bonds in the film matrix to understand interactions between plasticizers and the film matrix. We hypothesised that the number of O–H bonds would decrease with higher concentrations of each plasticizer because they could reduce hydrogen bonds. Furthermore, we conducted a cytotoxicity test to confirm that the films were non-toxic. We discussed an economic perspective to gauge the viability of biofilms in industry.

We concluded that the PEG 3% w/v films displayed the highest TS and lowest number of O–H bonds. The cytotoxicity test showed that our films were non-toxic. Even though the production costs of gelatin films are more expensive than oil-based plastics, our work remains important for considering the viability of green packaging and exploring novel approaches to optimize biofilm engineering.

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RESULTS

Our films were made from store-bought gelatin powder, water and plasticizers, PEG or glycerol. We casted the mixture and left it to dry overnight in an oven. The TS and EAB by tensile testing and the number of O–H bonds formed by fTIR revealed their comparative mechanical and chemical characteristics. We also performed an MTT assay to determine whether the films were non-toxic and considered the economic viability of commercially produced films.

Mechanical Properties

We observed a visible difference in the texture and colour of the different films **(Figure 1)**. There was a layer of oil on the surface of all films. The pure gelatin films were transparent with a noticeable yellow tint. They were brittle with many surface fractures and curled around the edges of the petri dish. The 1% and 2% w/v glycerol plasticized films were less tinted, but they were still relatively brittle. Air bubbles in the 3% w/v glycerol film made the film cloudy. Emulsification of the PEG mixture caused the 2% w/v PEG film to be translucent. The 1% w/v PEG film was the most transparent and visually appealing. Surface grooves were present on all PEG films. They were most pronounced on the 3% w/v PEG film.

We measured the thickness of the films using an electronic micrometre. The glycerol films showed a relatively small range between 0.101 to 0.176 mm **(Table 1)**. While the PEG films showed greater variation with a range of 0.274 to 0.577 mm. All PEG films were thicker than glycerol films and all glycerol films were also thinner than pure gelatin films.

The pure gelatin films have an average TS of 3.65 MPa **(Figure 2)**. The TS increased from 2.32, 7.58, to 10.30 MPa at PEG concentrations 1%, 2% and 3% w/v. The TS decreased from 7.30, 1.69, to 1.44 MPa. We found a statistically significant difference in the types of plasticizers ($F(1) = 69.98$, $p < 0.0001$), and the concentration of plasticizers used ($F(2) = 3.91$, $p = 0.0493$). This was a significant interaction ($F(2) = 116.53$, $p < 0.0001$). The TS was significantly increased by adding 1% and 2% w/v PEG to biofilms ($p = 0.0001$) and continued to increase when increased to 3% w/v PEG ($p = 0.0169$). It is suggested that adding progressively higher concentrations of PEG will have notable advantages to its strength. This can be due to changes in bonding between PEG and the gelatin polymers. TS increased significantly from 1% to 2% w/v glycerol ($p < 0.0001$), but it did not increase when 2% w/v concentration was increased to 3% w/v glycerol ($p = 0.9989$). It implies that the strength will not decrease at higher glycerol

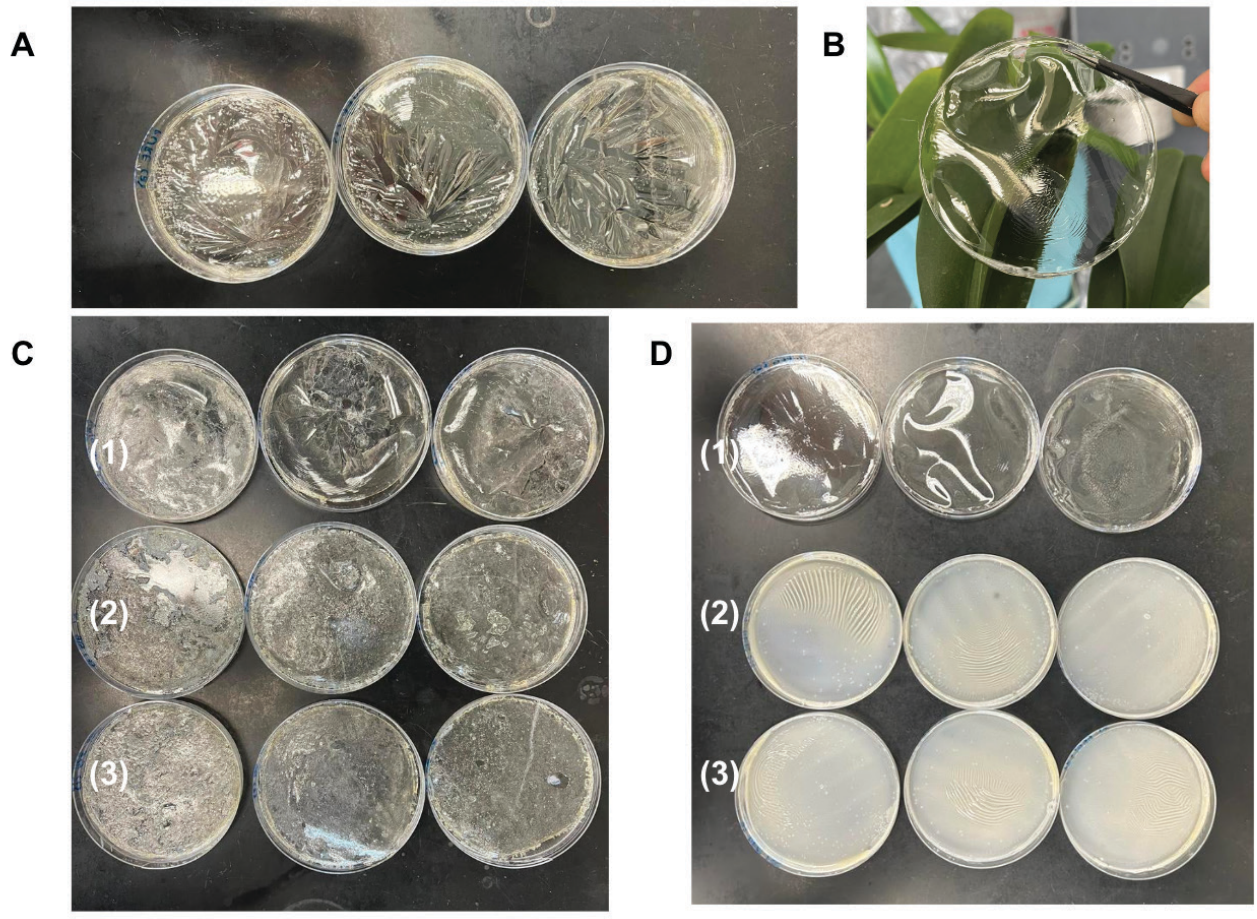

Figure 1: Qualitative observations of the dried biofilms. Gelatin films were dried in Tryptic Soy Agar Petri dishes at 60 °C in an oven for 24 hours. Photographs of dried **(A)** pure gelatin, **(B)** Film of 1% w/v PEG concentration against a houseplant to demonstrate transparency, **(C)** glycerol plasticized, and **(D)** PEG plasticized films. In C-D) concentration (w/v) is indicated on each row where (1) = 1%, (2) = 2%, and (3) = 3%.

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Type of plastic	Thickness (mm)	Tensile Strength (MPa)	Elongation at break (%)
pure gelatin	0.185 ± 0.0137	3.65 ± 0.115	4.87 ± 0.161
1% w/v PEG	0.101 ± 0.00216	2.32 ± 0.0918	0.56 ± 0.0573
2% w/v PEG	0.174 ± 0.030	7.58 ± 1.09	4.00 ± 0.649
3% w/v PEG	0.132 ± 0.00939	10.30 ± 1.22	4.10 ± 0.278
1% w/v glycerol	0.274 ± 0.00141	7.30 ± 0.0735	1.70 ± 0.376
2% w/v glycerol	0.390 ± 0.167	1.69 ± 0.0896	1.73 ± 0.417
3% w/v glycerol	0.557 ± 0.0328	1.44 ± 0.122	3.18 ± 0.646

Table 1: Mechanical properties of gelatin films (n=3). Average values for the thickness, TS, and elongation at break of different biofilms. Uncertainties were derived from the standard deviation taken to three significant figures following the mean.

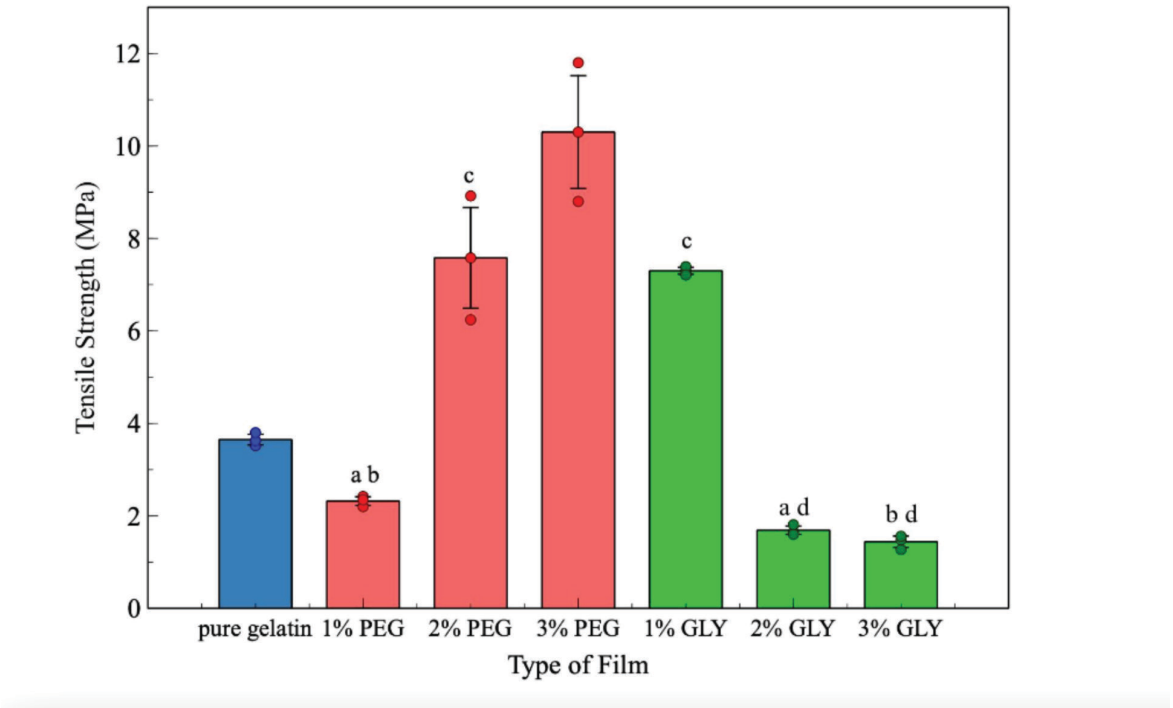

Figure 2: Tensile strength (MPa) of different concentrations of plasticizer in gelatin films (n=3). Bar chart showing the average tensile strength of pure gelatin film, PEG plasticized films (1%, 2%, 3% w/v), and glycerol plasticized films (1%, 2%, 3%. w/v). Samples of gelatin films were tested using a tensile machine at a crosshead speed of 10 mm/min. Error bars present standard deviation. Two-way ANOVA, plasticizer: $p < 0.0001$, concentration: $p = 0.0493$, plasticizer*concentration, $p < 0.0001$. Bars annotated with the same letter (eg. b and b on 1% PEG and 3% GLY) represent values that are not significantly different ($p < 0.05$).

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concentrations, reaching a plateau. 3% w/v PEG has higher TS than 3% w/v glycerol ($p < 0.0001$). PEG plasticizer is more effective in increasing the load than what the gelatin films can withstand.

The pure gelatin film had a high elongation at a break of 4.87% elongation from its original length **(Figure 3)**. There were large differences between the EAB of 1% and 2% w/v PEG concentration, increasing from 0.56% to 4.00%. At 3% w/v PEG, there was a small increase to 4.10%. The EAB of glycerol films, showed a gradual increase, from 1.70%, 1.73% to 3.18%. The types of plasticizers used ($F(1) = 6.78$, $p = 0.0231$) and the concentrations of plasticizers ($F(2) = 32.09$, $p < 0.0001$) are the main effects that impact EAB. There was also a significant interaction effect ($F(2) = 14.29$, $p = 0.0007$). The EAB increased significantly for 1% and 2% w/v PEG ($p = 0.0001$), but it did not increase when concentration was increased to 3% w/v ($p = 0.9999$). We propose that the film matrix is saturated with PEG molecules at 2% w/v. Further increases in the concentration did not increase flexibility as the plasticisers were in excess. There were no significant increases in EAB between 1% and 2% w/v glycerol films ($p = 1.0$), and 2% and 3% w/v ($p = 0.663$) as the glycerol molecules could have formed hydrogen bonds to the gelatin polymer upon its initial addition at 1% w/v. Higher concentrations of glycerol will not increase the number of hydrogen bonds, so EAB was not impacted. While there was 2% w/v PEG was superior to glycerol films at the same concentration ($p = 0.0033$), there was no difference between 3 w/v% glycerol and PEG films. The concentration of each plasticizer only increases EAB to a certain extent.

We then compared our results to commercial films, namely commercial Kraft paper and LDPE. Commercial Kraft paper had a TS of 16.11 MPa and an EAB of 1.53%. LDPE had a TS of 10.77 MPa and an EAB of 11.28%. Generally, Kraft paper and LDPE overall had higher TS than our films, indicating that they could withstand higher loads. Conversely, most of our films have a higher EAB than Kraft paper but not LDPE. This suggests that our films surpassed the flexibility of Kraft paper.

We plotted tensile stress against tensile strain for the 2% w/v concentration of each plasticizer against the pure gelatin film, commercial Kraft paper and LDPE **(Figure 4)**. For each film, there was an initial linear relationship. Within this elastic region, the films had not undergone plastic deformation. When the load reduces, the films return to their original shape (11). The ductile properties were measured by EAB, representing the extent our films deformed at fracture. In ductile films, the strain increases faster than stress beyond the elastic region, exhibiting plastic behaviour (11). A large strain fracture demonstrates ductile properties. Kraft paper showed behaviour typical of brittle materials. These materials often fracture at the end of the elastic region. LDPE peaked at a point that represents its ultimate TS **(Figure 4)**. It coincided with our observations of necking–the cross-section narrowed and stretched before breaking. As tensile stress is calculated using the original cross-sectional area after necking, tensile stress decreases before fracture (11). A material with high ductility and high strength has high toughness (11). The stress-strain analysis supports PEG films' superior toughness **(Figure 4)**. It showed a balance of high TS and EAB **(Figure 2)**. The pure gelatin films and 2% w/v glycerol

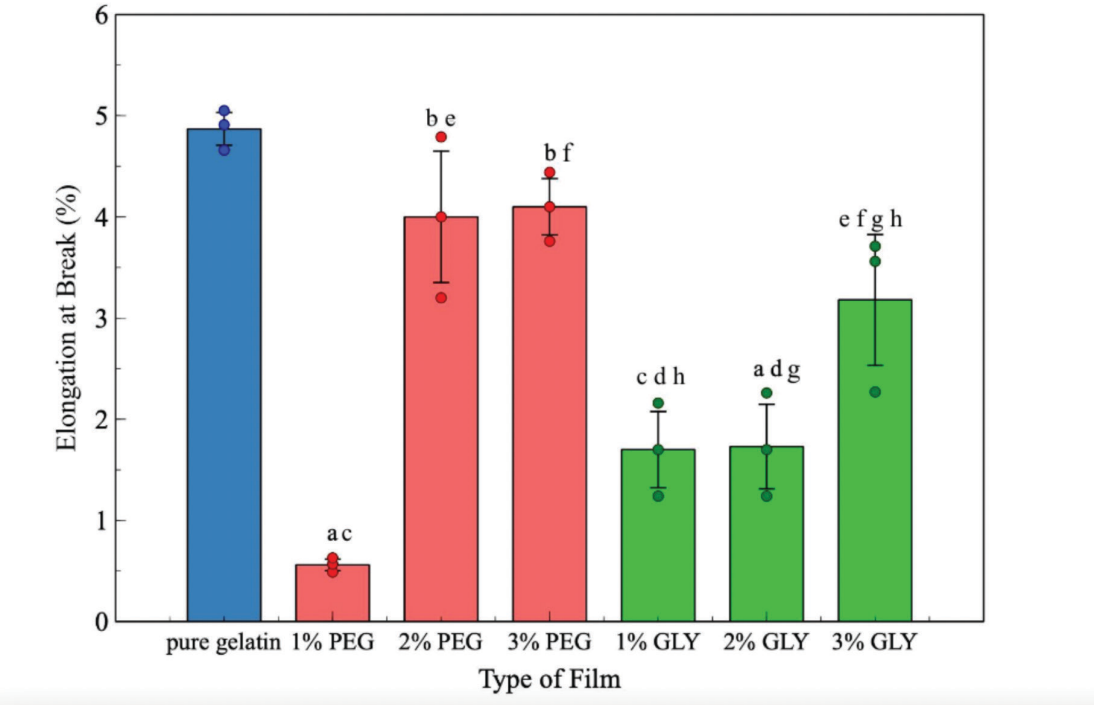

Figure 3: Percentage elongation at break of different concentrations of plasticizer in gelatin biofilm (n=3). Bar chart showing the average elongation at the break of pure gelatin film, PEG plasticized films (1%, 2%, 3% w/v), and glycerol plasticized films (1%, 2%, 3% w/v). Samples of gelatin films were tested using a tensile machine at a crosshead speed of 10 mm/min. Error bars present standard deviation. Two-way ANOVA, plasticizer: $p = 0.0231$, concentration: $p < 0.0001$, plasticizer*concentration, $p = 0.0007$. Bars annotated with the same letter (eg. b and b on 2% PEG and 3% PEG) represent values that are not significantly different ($p < 0.05$).

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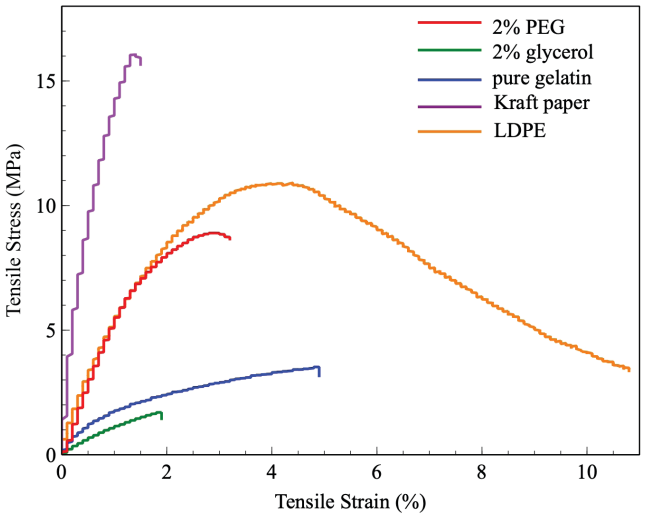

Figure 4: Stress-strain analysis. Tensile strain against tensile stress of pure gelatin films, 2% w/v glycerol and PEG films, LPDE and Kraft paper. Film samples were tested using a tensile machine at a crosshead speed of 5 mm/min.

films ruptured with lower stress than 2% w/v PEG films. The 2% w/v glycerol films underwent plastic deformation as the curve continued beyond the initial linear region. They also have a similar curve following LDPE up to the 2% strain.

Chemical Characterization

We characterised the chemical composition of the 2% w/v formulations by FTIR, which is a spectroscopic technique that causes different types of bonds within a compound to undergo asymmetric molecular vibrations by changing its dipole moment by absorbing infrared radiation (15). This forms a series of peaks corresponding to the vibrations of bonds at specific wavenumbers. The intensity of the peaks reveals the relative abundance of the bonds and the level of change in the dipole moment (16). We targeted the O–H absorption peak because the film matrix comprises hydrogen bonds between gelatin, water and plasticizers. The O–H bonds within the structure are indicated by the broad absorption peaks at approximately 3400 cm^{-1} **(Figure 5)**. Our results revealed a shift in the peak position to the left in plasticized films compared to the pure gelatin film, suggesting that the plasticizers formed bonds within the matrix. The 2% w/v PEG biofilms had the lowest absorption at 3400 cm^{-1} **(Figure 5)**. It suggests that there were fewer hydrogen bond interactions between plasticizers with water and gelatin polymers. The 2% w/v glycerol biofilms had the next lowest absorption, and gelatin had the broadest absorption peak **(Figure 5)**. The highest number of hydrogen bonds formed in the pure-gelatin matrix.

Cytotoxicity

A cytotoxicity analysis was completed to test whether the films were non-toxic and food-safe, reinstating ideas for potential applications as food packaging. According to regulatory measures published by the Food and Drug Administration (FDA), food contact substances (FCS) such as plastic take-out boxes are recommended to undergo genetic toxicity testing before being deemed as food safe (17). The

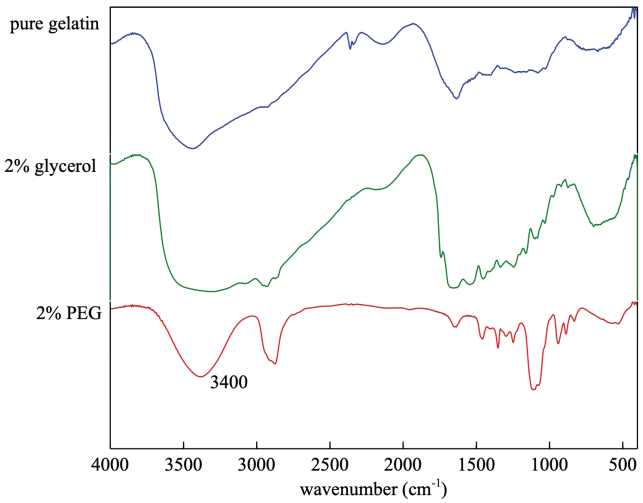

Figure 5: FTIR of gelatin biofilms. FTIR spectrum of pure gelatin film, 2% w/v glycerol film and 2% w/v PEG film. Samples of gelatin films were tested with a wavenumber of 400 cm^{-1} to 4000 cm^{-1}.

FDA suggests the usage of a genotoxicity assay to test for carcinogenic compounds present in the FCS, but it is also stated that an alternate test deemed appropriate may also be used (17). Cytotoxicity refers to the extent to which a substance may cause damage to a cellular body, as opposed to genotoxicity, which only alters the genetic material within the cell (18). The cytotoxicity MTT assay allowed for a colorimetric analysis to examine whether a substance will lead to decreased cell proliferation or necrosis, thereby giving an indicator of the substance's safety (19). A549 cells were derived from the pulmonary carcinoma tissue of a Caucasian male and are commonly used for a range of clinical trials and preliminary drug tests (20). Cell viability was obtained by testing 3 samples of pure gelatin films, 3% w/v PEG, and 3% w/v glycerol plasticized films. The average cell viability was 96.3%, 84.1%, and 88.5%, respectively **(Figure 6)**. All the bioplastics were determined to be non-toxic and food-safe because the cell viability was above the threshold of 75%, a value from the modified procedures of Chen *et al.* that we adopted **(Figure 6)** (21).

DISCUSSION

In regards to mechanical properties, glycerol decreased TS because polymer-polymer bonds and polymer-water bonds are replaced by polymer-glycerol bonds, disrupting the regular crystalline matrix (22). It increased the chain mobility of adjacent gelatin strands by increasing chain movement which promoted polymer movement and improved the extension of the film (23). The downward trend of TS with increasing concentrations of glycerol in the biofilms was also observed in a similar study on catfish skin gelatin films (13). On the other hand, the upward trend of TS in PEG films was unexpected. Ardianisa and Suprayitno reported that increasing concentrations of PEG decreased TS (24). This contradicts our findings. Bai *et al.* suggested that one possible contributor to increased TS could be the increased viscosity of casting solutions (25). While we did not observe any visible changes in viscosity, rheology could be used to quantify a possible relationship between TS and viscosity. A shear stress test would be an appropriate method to determine the

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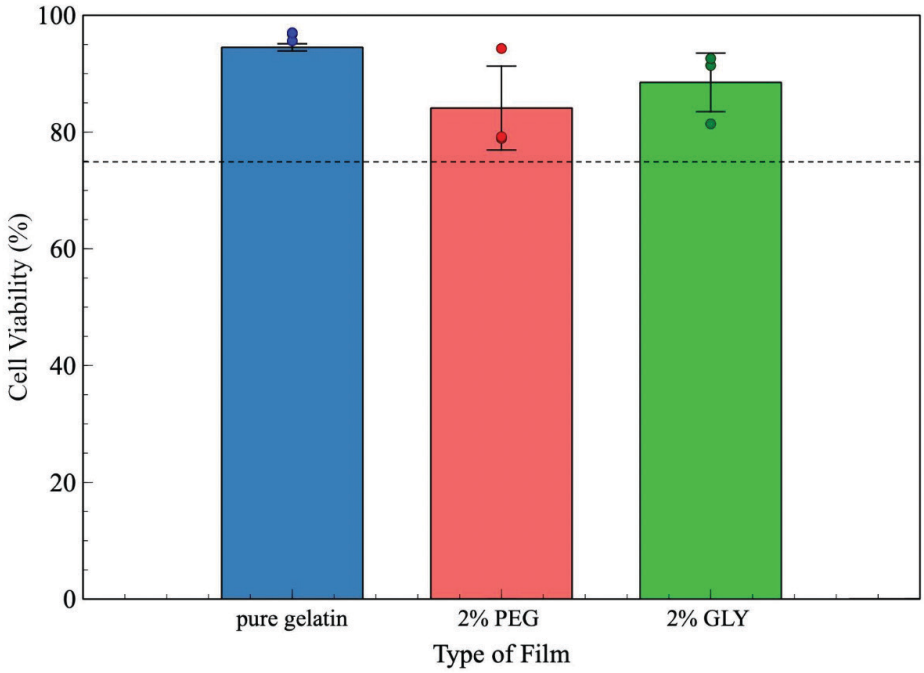

Figure 6: Cell viability (MTT Assay) of pure gelatin, PEG, and glycerol bioplastic films (n=3). Cell viability of A549 cells after exposure to pure gelatin, 3% w/v PEG and 3% w/v glycerol films for 24 hours. Viability was measured using an MTT assay and compared to a no-biofilm control. The dotted line represents the threshold of viability in food safety.

elasticity of each material.

PEG films had a significantly higher EAB value compared to gelatin films. There were similar findings of a large peak in EAB for 2% w/v in PEG-plasticized sago starch films (26). Literature indicated a decrease in EAB after 2% w/v because of phase separation. We anticipate that elongation at the break would increase between lower intermediate concentrations such as 0.25% and 0.5% w/v and reach a plateau. It would eventually decrease at higher concentrations.

The lowest number of O–H bonds between the gelatin polymer strands and the plasticizer formed caused increased stretchability **(Figure 5)**. PEG acts as an external plasticizer. As a result, it does not covalently bond to gelatin polymer strands. Instead, it forms weak second-order bonds with polymer chains, allowing it to remain mobile between the polymer chains (26). It can easily separate from the film, which can explain the oil precipitation we observed. Glycerol, in contrast, behaves as an internal plasticizer, forming hydrogen bonds with the gelatin polymer backbone (26). Glycerol is static between adjacent polymer chains. Moreover, glycerol is a small molecule with three O–H functional groups, whereas PEG is larger with only two O–H functional groups (27, 28). Glycerol is thus able to form more hydrogen bonds to gelatin than PEG. Together, PEG's structure and bonding account for the small absorption peak and large elongation at the break value.

Compared with conventional plastic like low-density polyethylene (LDPE), which has a TS of 10.77 MPa, has a 0.47 MPa difference with 3% w/v PEG film. When considering EAB, LDPE demonstrates 11.28% elongation while gelatin biofilms exhibit 4.10% elongation (at 3% w/v PEG). Another alternative to oil-based plastics is Kraft paper. It displays an elongation at a break of 1.53% and a TS of 16.11 MPa. Its EAB is considerably lower than our gelatin films and LDPE. Despite this, paper bags are used by stores worldwide, capturing a 22% share of the global market in 2023 (29). We argue that gelatin films remain a viable alternative to LDPE despite their low EAB. We can increase the comparative strength of PEG films by increasing the plasticiser concentration.

It is worth noting that the intrinsic film properties limited our investigation. When peeling the films from the petri dish and cutting them to produce lengths for tensile testing, they were fragile and broke during handling. This results from the strong hydrogen bonds between gelatin polymers and water. By changing temperature, we can aid the restructuring of gelatin and enhance film properties. Gelatin solutions exhibit a vitrification mechanism as they set. They transition into a glass or a non-crystalline amorphous solid (30). High temperatures, such as 60°C, coincide with the melting point of gelatin. At 60°C, gelatin remains as a liquid while water is driven off. Aguirre-Álvarez, *et al*. (30) suggested that the heightened temperature builds an amorphous zone, whereas a lower temperature such as 20°C would promote the formation of organised junction zones. This lack of a continuous crystalline network may have made samples more brittle. Fragile films tended to produce shorter-length films for testing. Still, the averaged values of TS and EAB have relatively low standard deviations, representing a low spread of data and considerable reliability. However, more research is needed to determine an ideal drying temperature within a reasonable drying period to strengthen the films.

Different formulations of gelatin will change the features of the films too. The extraction source of gelatin and the bloom value are two factors. Bloom is a measure of the strength and firmness of gelatin. The store-bought gelatin we used did not include a Bloom Index. Most store-bought gelatin powders

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ranged between 50 to 300 Bloom (31). A higher bloom index correlates with stronger mechanical properties. Said and Sarbon (32) demonstrated that poultry-based gelatin films performed better than mammalian and marine-based gelatin films. Procuring gelatin powder from a designated source and of a specific Bloom index will help standardise the features of the film.

From an economical perspective, the gelatin biofilms were manufactured at a base cost of 1.02 USD per film, neglecting the cost of plasticizers. Scaling up the production, we discovered that PEG is currently valued at 8.80 USD per kilogram at ECHEMI (33). Glycerol is priced between 7.00 and 11.00 USD/kg (34). Gelatin is presently priced at 4.70 USD/kg (35). By adding the costs of plasticizers, the total cost of producing PEG biofilms amounts to 13.50 USD per kilogram and producing glycerol biofilms is estimated to be 13.70 USD per kilogram (calculated as an average of 7.00 and 11.00 USD). Compared to manufacturing oil-based plastics, which is approximately 1-2 USD per kilogram, the production costs of gelatin biofilms are significantly more expensive (36). To reduce production costs, we suggest sourcing gelatin from marine and poultry waste, facilitating a circular economy.

In conclusion, 3% w/v PEG made the most desirable film. It outperformed other PEG films and 3% w/v glycerol films regarding TS. It had a TS of 10.30 MPa and an EAB of 4.10%, making it more flexible than commercial Kraft paper. At 2% w/v PEG, films had the smallest peak at 3400 cm^{-1} on the O–H group absorption peak, contributing to greater mobility between gelatin strands. These properties are promising steps in developing an alternative in packaging and other non-biodegradable plastics.

MATERIALS AND METHODS

Packaged unflavoured Robertson's gelatin powder was purchased from the local supermarket. Glycerol, with a molecular weight of 200 g/mol (PEG200) plasticizers and distilled water were provided by the Chinese University of Hong Kong (CUHK).

Gelatin Plastic Film Preparation

PEG and glycerol-plasticized gelatin films were prepared following the production process reported by Ardianisa and Suprayitino with modifications (24). 9 g of gelatin was weighed in a weigh boat on an electronic balance (Denver Instrument Company, Cat# AA-200). The gelatin was dissolved in 180 mL of distilled water and heated at 60oC for 15 minutes. PEG and glycerol were added to 60 mL of the gelatin-water mixture to make up 1%, 2% and 3% w/v concentrations. 20 mL of the solution was measured and poured into a petri dish containing tryptic soy agar (TSA). Petri dishes were placed in an oven at 60oC for 24 hours. A control sample without a plasticizer was also prepared.

Tensile Testing

The biofilms' TS and EAB are tested using a tensile machine (TOHNICHI) by uniaxial tensile tests. The thickness of the films was measured using an electronic micrometre (Kangalu, Cat#RZ0011B). Films were cut into strips with dimensions 10 mm by 50 mm using a cutting mold. The machine was set to a crosshead speed of 5 mm/min, and each sample was tested at least once following the GB/T 1040.3-2006 standard. Silicon plastics were stuck at the ends of the film using double-sided tape to increase grip during tensile testing. A two-way ANOVA test was used to analyse the relationship between varying concentrations of PEG and glycerol and against each other with a 5% significance level.

FTIR Analysis

The infrared spectra of the films were analysed by a Fourier Transform Infrared Analyzer (FTIR Alpha Spectrometer) device with a range of wavenumbers from 4000 cm^{-1} to 400 cm^{-1}. The biofilms were fit to the film holder. An average of 24 scans at 4 cm^{-1} resolution were taken.

MTT Assay Cytotoxicity Test

Cytotoxicity of the films was determined using an MTT Assay using a modified leaching liquid method documented by Chen *et al.* (21). A459 cells (ATCC, Cat# CCL-185) were used. Cell suspensions (1×10^4 cells/cm^3) were seeded in 96-well microtiter plates for 24 hours, with 5mg of each film and without in control experiments. The cells adhered to agar gel plates before the addition of films. After 24 hours, the MTT reagent (5 mg/mL) was added and incubated at 37 °C for 4 hours. After incubation, the media was replaced with 200 µL of dimethyl sulfoxide minimum 99.5% (DMSO) solution. The absorbance values were measured at 540 nm with a multi-plate reader (Labexim Products LEDE-TECT 96). Cytotoxicity, represented by a viability percentage, was calculated by the following equation.

Cell viability (%) = (Absorbance of sample ÷ Absorbance of the control group) × 100

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