Characterization of Biofilms Based on Agar, Gelatin, and Cornstarch Incorporating Glycerin as a Plasticizer

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ABSTRACT

This study explores the development and characterisation of biodegradable biofilms utilising agar, gelatin, and cornstarch as natural polymer bases, each integrated with 20% glycerin as a plasticiser to enhance flexibility and reduce brittleness. Preparation involved thermal processing (boiling) for a duration of 15–20 minutes. Agar solutions were boiled for 15 or 20 minutes before casting. All cast films were then left to dry for a few days at room temperature (20–25°C) until fully set. The resulting movies were systematically profiled for important performance parameters: moisture content (determined by gravimetric analysis post-drying), solubility (measured by mass loss post-immersion in water), biodegradability (measured by environmental degradation assays), and mechanical strength (analysed using tensile testing for stress-strain characteristics). Gelatin-glycerin films exhibited the lowest moisture content (15.38-16.09%) and highest tensile strength (6.34 MPa) with remarkable elongation at break (156.45%), indicating their strong potential for flexible wrapping, cosmetic layers, or biomedical scaffolds. Agar-based films exhibited balanced mechanical properties (4.75 MPa, 52.95% elongation), high water solubility (42.1-52.08%), and moderate moisture retention, making them suitable for edible coatings or semi-rigid packaging. Cornstarch films, though naturally biodegradable, demonstrated lower tensile strength (2.54 MPa) and reduced solubility and water absorption over time, making them suitable for disposable rigid biofilms. Overall, the incorporation of glycerin effectively improved plasticity across all formulations, and the data underscores the significance of polymer selection and thermal processing in tailoring biofilms for sustainable applications in food packaging, personal care, and eco-friendly materials.

Keywords — Water absorption; Mechanical; Moisture Content; Sustainable; Packaging

1.0 INTRODUCTION

The global reliance on petroleum-based plastics has led to escalating environmental concerns, particularly due to their persistence in ecosystems and their contribution to pollution. As sustainability awareness grows, the development of biodegradable alternatives has gained significant momentum. Biopolymer-based films derived from natural sources offer a promising solution, especially when formulated from agro-waste and renewable materials. Although these plastics significantly support the global economy, generating billions of dollars, their resistance to degradation poses a significant environmental challenge, resulting in severe ecological problems (Ahmad, 2018).

Agar, gelatin, and cornstarch were selected to represent the three major classes of biodegradable biopolymers (plant polysaccharide, animal protein, and seaweed polysaccharide, respectively) to facilitate a comprehensive comparative

study of how material source influences the mechanical properties, water sensitivity, and application potential of thermally processed films, ranging from low-cost, rigid packaging to highly flexible, elastic scaffolds. Agar, a polysaccharide extracted from red algae, forms transparent and flexible films with high solubility. Gelatin, a protein derived from collagen, provides excellent mechanical strength and elasticity. Cornstarch, a carbohydrate-based polymer, offers rigidity and water resistance but often requires plasticizers to improve flexibility.

Glycerin is widely utilized as a plasticizer, humectant, emollient, and solvent in food flavoring and coloring. Being highly hygroscopic, it can be incorporated into film-forming solutions to decrease film brittleness (Viera, 2011). Glycerin is commonly incorporated to enhance the mechanical properties and reduce brittleness of biofilms. Its compatibility with hydrophilic polymers makes it ideal for improving film flexibility and moisture retention. In this study, glycerin was incorporated at a fixed concentration of 20% (w/w) based on the total polymer mass across all three biopolymer formulations.

This study aims to develop and characterize biofilms based on agar, gelatin, and cornstarch incorporating glycerin, focusing on their moisture content, solubility, tensile strength, biodegradability, and surface morphology. By comparing the effects of polymer type and boiling duration, which significantly influence the molecular structure and density of the film, this research seeks to identify optimal formulations for sustainable packaging applications and contribute to advancing circular economy principles.

2.0 THEORY/LITERATURE REVIEW

Biopolymer-based biofilms, particularly those derived from agar, gelatin, and cornstarch, offer promising alternatives to petroleum-based plastics due to their biodegradability and film-forming capabilities. Agar's gel-forming polysaccharide structure supports high solubility and moderate strength, while gelatin's protein matrix provides superior elasticity and rapid environmental breakdown. Cornstarch, though cost-effective and biodegradable, requires plasticizer enhancement to overcome brittleness. Glycerin improves flexibility by disrupting hydrogen bonding and increasing polymer mobility. The role of polymer type and plasticizer concentration in tailoring mechanical, moisture, and degradation properties for sustainable biofilm applications (Fathiraja, 2022). However, in this study, the glycerin concentration was consistently fixed at 20% to isolate the effects of polymer type and boiling duration.

3.0 MATERIALS

In this experiment, agar (Food Grade, extracted out of algae (Japanese origin), gelatin (Food Grade, extracted out of fish), and cornstarch (Regular Food Grade) were dissolved in distilled water separately and mixed with 20% glycerin, vegetable-based, 99.5% purity (Food Grade), as a plasticizer. All solutions were prepared using an analytical balance and heated using a magnetic stirrer.

4.0 EXPERIMENTAL

The biofilms were prepared by dissolving each of the biopolymers separately agar (AG), gelatin (GG), and cornstarch (CG) in 100 mL of distilled water with glycerin used as the plasticizer at a constant proportion of 20% of the total polymer weight. All mixtures were heated using a magnetic stirrer hot plate to 60°C to allow complete dissolution. The solutions obtained were then boiled for 15 or 20 minutes to determine the effect of thermal processing time**.** Lastly, the warm solutions were solution-cast onto a silicone mould with 20mL of solution and left to dry

completely at room temperature (20–25°C) over several days.

4.1 Biofilm Formulation

Biofilm is produced by mixing agar (20%:20%), gelatin (50%:20%), and cornstarch (50%:20%). This mixture is suitable for each different material. Each formulation incorporates glycerin at consistent concentration of 20% based on the total polymer mass, serving as a plasticizer to enhance flexibility and reduce brittleness as shown in Fig. 1.

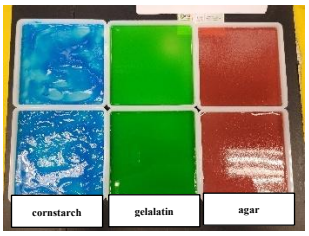

Fig. 1: Biofilm made from Cornstarch, Gelatin and Agar

To investigate the effect of thermal processing, the biofilm is subjected to two boiling durations 15 minutes and 20 minutes before casting. The variable aims to assess how boiling time influences the physicochemical characteristics of the film, including structural uniformity and mechanical strength.

4.2 Characterization Biofilm

Various physical and mechanical parameters were examined to evaluate the quality and functionality of the biofilms produced from agar, gelatin, and cornstarch with glycerin. Moisture content and water/alcohol solubility tests were conducted to determine the films' hydration and dissolution behaviours. Water absorption was assessed to determine the film's moisture-retention capacity. Biodegradability tests provided insight into the environmental impact of each formulation. Tensile strength analysis measured the films' mechanical durability which was recorded for comparative evaluation.

4.2.1 Moisture Content

Boiling time is crucial for modifying the molecular structure and moisture characteristics of biopolymer-based films. The content of moisture was calculated by weighing the sample of biofilm before and after 24 hours of oven drying at 85°C for 24 hours as shown in Table I. The moisture percentage was determined by using formula 1:

$$Moisture\ Content\ (\%) = ((\frac{(W1 - W2)}{W1}) \times 100)$$

4.2.2 Solubility of Water and Alcohol

Solubility was determined by weighing dried biofilm samples (W1) as indicated in Table II, immersed in 50 mL of distilled water and 3 mL of ethanol at room temperature after 24 hours, then filtering and air-drying the residue after another 24 hours (W2). The choice of solvents (both distilled water and ethanol) represents two different polarity conditions: water is a hydrophilic/aqueous environment (relevant to food packaging or edible use), and ethanol is a lipophilic/alcohol-rich environment (relevant to pharmaceutical or cosmetic use), to compare the dissolution behaviour of both biofilms comprehensively. The solubility (%) was determined based on formula 2:

$$Solubility\ (\%) = ((\frac{(W1 - W2)}{W1}) \times 100)$$

4.2.3 Water Absorption

Results indicate the absorption behaviour of biopolymer films made from agar, gelatin and cornstarch mixed with glycerin at 15 and 20 minutes of exposure, as indicated in Table III. The absorption of water was measured by drying the biofilm samples at room temperature for 24 hours

to record their initial weight (W1). The samples were again immersed in distilled water overnight, filtered and reweighed (W2). The percentage of absorption was determined by formula 3:

$$Water\ Abrp.(\%) = (\left(\frac{(W1 - W2)}{W1}\right) \times 100)$$

4.2.4 Biodegradability Test

The biodegradability of agar (AG), gelatine (GG), and cornstarch (CG) biofilms containing glycerine was evaluated in soil and wet sand environments, and Table IV showed different degradation patterns. To mimic natural soil biodegradation conditions typically found in composting or landfill settings, soil was used, and to mimic a moisture-saturated environment with specific microbial communities, wet sand (saturated with river water) was used, allowing a comparative evaluation of biodegradation behaviour in two environmentally relevant media. Biodegradability was evaluated by weighing dry biofilm samples (W1) and burying them in soil or wet sand for 1 week. The rest was then cleaned, dried and reweighed (W2). The percentage of biodegradation was calculated using formula 4:

$$Biodegradable(\%) = ((\frac{(W1 - W2)}{W1}) \times 100)$$

This study employs the acronym AG, GG, and CG to represent the three different biofilm formulations, all of which include Glycerin as a plasticizer. AG is the abbreviation of Agar-Glycerin formulation, GG is the Gelatin-Glycerin film, and CG is the Cornstarch-Glycerin biofilm.

5.0 RESULTS AND DISCUSSION

5.1 Moisture Content

Table I: Biofilm Moisture Content (%) Data analysis

Sampel	Data		
	W1	W2	Moisture Content%
AG 15	0.55	0.37	32.73
AG 20	0.84	0.6	28.57
GG 15	0.87	0.73	16.09
GG 20	1.56	1.32	15.38
CG 15	0.61	0.5	18.03
CG 20	0.84	0.68	19.04

Agar-based biofilms' moisture content decreased slightly from 32.73% after 15 minutes of boiling to 28.57% at 20 minutes. Agar's gel network tightens during prolonged boiling, potentially reducing its water-holding capacity by strengthening polymer interactions (Amira, 2023). This suggests that extended boiling time produces denser agar films with lower moisture affinity.

Gelatin films, on the other hand, showed the lowest moisture content overall, recording 16.09% at 15 minutes and 15.38% at 20 minutes. The decrease is marginal but reflects the compact nature of gelatin's protein matrix. Extended boiling may promote tighter crosslinking (Walid, 2021), further limiting water uptake. Cornstarch films recorded slightly increasing moisture levels of 18.03% at 15 minutes and 19.04% at 20 minutes. This suggests that longer boiling may expand starch granules or expose more hydrophilic sites, increasing water retention (Jatin, 2019).

Overall, gelatine films were the least moisture-retentive, regardless of boiling time; agar films were more sensitive to thermal treatment when plasticised, especially in terms of moisture absorption (Rusli, 2016). While starch-based films can

become more moisture-absorbent with thermal modification, depending on the extent of gelatinization and matrix porosity (Budsaereechai, 2021). These findings highlight how thermal processing time during film formation can fine-tune moisture behaviour across different biopolymers.

5.2 Solubility in water and alcohol

Table II: Solubility in Water and Alcohol (%) Data Analysis

Sampel	Solubility of Water		
	W1	W2	Solubility %
AG 15	0.38	0.22	42.1
AG 20	0.48	0.23	52.08
GG 15	0.57	0.44	22.8
GG 20	1.08	0.83	23.14
CG 15	0.45	0.33	26.66
CG 20	0.35	0.30	14.23
Sampel	**Solubility of Alcohol**		
AG 15	0.58	0.38	34.48
AG 20	0.41	0.25	39.02
GG 15	0.61	0.58	4.91
GG 20	0.92	0.83	9.78
CG 15	0.35	0.23	34.28
CG 20	0.78	0.75	3.84

The solubility profiles of agar (AG), gelatine (GG), and cornstarch (CG) biofilms in water and alcohol reveal distinct time-dependent behaviours influenced by polymer structure and glycerine plasticization. AG films exhibited the highest solubility in both solvents, increasing from 42.10% to 52.08% in water and 34.48% to 39.02% in alcohol over 15 to 20 minutes. This trend reflects agar's hydrophilic polysaccharide backbone and its susceptibility to matrix loosening, further enhanced by glycerine's facilitation of solvent permeation (Asma, 2016). GG films maintained low solubility across both media (water: 22.80–23.14%; alcohol: 4.91–9.78%), indicating a stable protein network with limited responsiveness, likely due to cross-linking that resists hydration and ethanol diffusion (Asma, 2016). CG films showed a marked decline in solubility over time (water: 26.66% to 14.23%; alcohol: 34.28% to 3.84%), suggesting retrogradation or crystallisation that restricts solvent access.

The discussion highlights how biofilm composition and exposure duration critically shape solubility behaviour. AG's increased solubility supports its use in time-sensitive applications such as edible wraps or drug carriers. GG's stability suits humid or alcohol-rich environments, while CG's declining solubility signals caution for long-term aqueous exposure. These findings reinforce Gholamreza et al. (2021), emphasizing the interplay of glycerine and polymer type in tailoring biopolymer performance for specific functional needs.

5.3 Water Absorption

Table III: Biofilm Water Absorption (%) Data analysis

Sampel	Data		
	W1	W2	Biofilm Water Absorption %
AG 15	0.72	1.68	133.3
AG 20	0.86	2.34	172.1
GG 15	0.77	13.91	171
GG 20	1.2	32.91	264
CG 15	0.73	1.51	106.8
CG 20	1.13	2.18	92.92

AG films showed consistently high absorption (146.67% to 153.33%), reflecting agar's hydrophilic polysaccharide structure and glycerines plasticizing effect (Rusli, 2016). GG films increased from 85.71% to 126.66%, suggesting a gradual loosening of the protein matrix and enhanced water uptake (Gholamreze, 2021). In contrast, CG films

declined from 172.72% to 166.66%, likely due to retrogradation or crystalline rearrangement that restricts water ingress (Gholamreze, 2021). The discussion highlights how biofilm composition and exposure time influence moisture responsiveness. AG and GG films are promising for biomedical or edible applications requiring high water absorption, while CG's behaviour suits applications requiring initial swelling ability with later structural integrity. These trends align with those of Gholamreza et al. (2021), emphasising the roles of plasticiser concentration and polymer type in shaping hydrophilic performance.

5.4 Biodegradable Test

Table IV: Biofilm Biodegradable Test (%) Data analysis

Sampel	Soil		
	W1	W2	Biofilm Biodegradable %
AG 15	0.62	0.26	58.06
AG 20	0.70	0.3	57.14
GG 15	0.78	null	0
GG 20	1	null	0
CG 15	0.93	0.58	37.63
CG 20	0.96	0.5	47.91
Sampel	**Sand**		
AG 15	0.73	0.3	58.9
AG 20	0.73	0.29	60.27
GG 15	0.78	null	0
GG 20	1.12	null	0
CG 15	0.68	null	0
CG 20	1.13	null	0

Fig. 2: Biodegradability Test Biofilm Before One Week in Soil and Sand

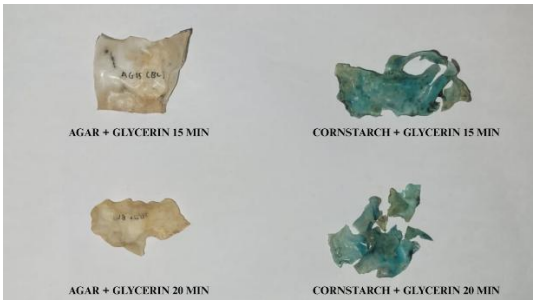

Fig. 3: Biodegradability Test Results of Biofilms Treated with Glycerin After One Week in Soil.

In soil, GG films showed complete decomposition within one week, indicated by "null" final weights, making them the most biodegradable. This high biodegradability is because gelatin is a protein-based matrix that is readily susceptible to rapid breakdown by the proteolytic microbial enzymes present in the soil AG films degraded moderately (58.06–57.14%) as a polysaccharide, agar is also biodegradable, but its gel-forming structure offers more structural integrity, leading to a slower degradation rate than the protein-based gelatin, while CG films showed lower biodegradability (37.63–47.91%), this lower performance is attributed to cornstarch's semi-crystalline nature. This arrangement and retrogradation possibility cause microbial enzymes to find it hard to penetrate the polymer chains and break them down, limiting access and resulting in the lowest rate of degradation. Visual observations (Figure 3) supported these findings, with AG films showing fragmentation and CG films retaining more structure, suggesting that exposure time of the film in the soil after one week

influences durability and microbial accessibility which can be seen in Fig. 3.

Fig. 4: Biodegradability Test Results of Biofilms Treated with Glycerin After One Week in Sand.

Under wet-sand conditions, GG and CG films again showed complete degradation, with "null" values indicating total breakdown rather than poor performance. AG films retained measurable residue but exhibited high biodegradability (58.9–60.27%), with more prolonged glycerine exposure accelerating deterioration (Figure 2). The saturated sand environment, rich in moisture from river water, likely enhanced microbial activity and enzymatic access, facilitating rapid decomposition. These results confirm that GG is highly suitable for applications requiring fast environmental breakdown, while AG offers stable yet effective biodegradability. CG's performance improves in moist conditions, suggesting its potential when paired with higher glycerine concentrations, as shown in Fig. 4.

It emphasizes the critical role of biofilm composition, glycerine concentration, and environmental moisture in determining biodegradation rates. Frequent rainfall and humid conditions during the soil test further accelerated microbial breakdown, especially for GG and CG films. These findings align with Gholamreza et al. (2021), highlighting how plasticizer integration and environmental factors shape biopolymer degradation and guide application-specific material selection.

5.5 Tensile Test

The mechanical performance of bioplastics formulated from agar (AG), gelatin (GG), and cornstarch (CG) with glycerin varies significantly due to differences in polymer structure and plasticizer interaction as shown in Table V.

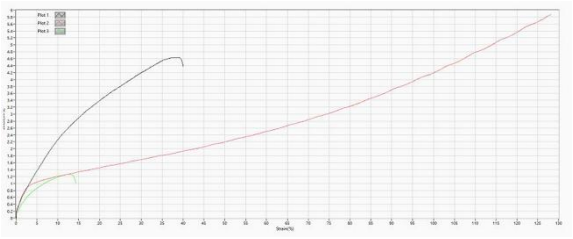

Fig. 5: Mechanical Performance Biofilm (Agar, Gelatin and Cornstarch) Boiling Point For 15 Minutes.

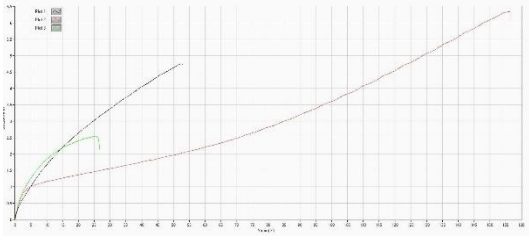

Fig. 6: Mechanical Performance Biofilm (Agar, Gelatin and Cornstarch) Boiling Point For 20 Minutes.

Fig. 7: Biofilm Before Tensile Test.

Fig. 8: Biofilm After Tensile Test.

Table V: Biofilm Tensile Test Data Analysis

Sampel	15 Minutes Boiling Point				
	Area (mm^2)	Peak Force (N)	T. Strength (MPa)	Elongation (%)	Yong's Modules (MPa)
AG 15	1.65	7.65	4.63	39.97	19.69
GG 15	2.55	14.96	5.87	128.13	2.85
CG 15	3.45	4.36	1.26	14.35	20.13
Sampel	**20 Minutes Boiling Point**				
AG 20	3.00	14.24	4.75	52.95	12.57
GG 20	5.40	34.24	6.34	156.45	2.64
CG 20	2.40	6.10	2.54	26.61	22.08

AG films demonstrated moderate tensile strength (4.63–4.75 MPa) and elongation (39.97–52.95%), balancing rigidity and flexibility. Agar's gel-forming polysaccharide backbone provides structural integrity, while glycerine disrupts intermolecular forces to reduce brittleness (Rusli, 2016).

GG films exhibited superior mechanical properties, with tensile strength ranging from 5.87 to 6.34 MPa and elongation from 128.13% to 156.45%, indicating high elasticity and softness. Gelatin's protein-based matrix, enhanced by glycerine, allows for excellent stretchability and moisture retention (Gholamreza, 2021). The low Young's modulus (2.85 MPa) confirms minimal stiffness, making GG ideal for cosmetic layers and flexible packaging.

In contrast, CG films showed the lowest tensile strength (1.26–2.54 MPa) and elongation (14.35–26.61%), with the highest stiffness (Young's modulus: 20.13–22.08 MPa), indicating brittleness and limited flexibility. Cornstarch's semi-crystalline nature resists plasticization, and glycerine alone cannot soften its matrix (Harussani et al., 2021). Overall, GG offers the best mechanical profile, AG provides moderate versatility, and CG suits rigid applications. These results underscore the need for tailored formulations to meet specific bioplastic performance demands.

5 CONCLUSION

This study demonstrates the potential of agar, gelatine, and cornstarch as biodegradable biofilm bases when plasticized with glycerine. Gelatine films exhibit superior mechanical strength and complete biodegradability, making them ideal for flexible, fast-degrading applications. Agar films offered balanced solubility and tensile properties, suitable for edible and semi-rigid packaging. Cornstarch films, while naturally biodegradable, showed limited flexibility and solubility, which is best suited for rigid disposables. The boiling duration influenced moisture content, water absorption, and surface morphology, highlighting the importance of thermal processing. These findings emphasise that polymer selection and glycerine integration are key to tailoring biofilms for sustainable packaging and environmental applications.

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